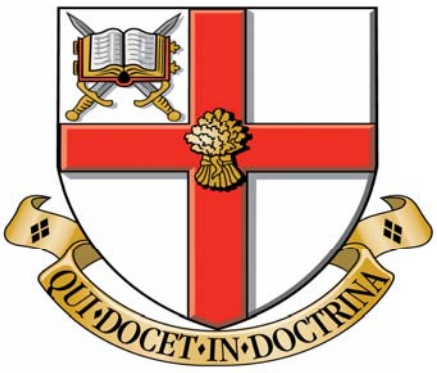

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Social and environmental influences on the welfare of zoo-housed spider monkeys (*Ateles geoffroyi rufiventris*)

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By Nick Davis

May 2009

Declaration

I hereby declare that this thesis is of my own composition and that all assistance has been acknowledged. The results presented in this thesis have not previously been submitted towards any other degree or for another qualification.

Nick Davis

TABLE OF CONTENTS

TABLES **i**

FIGURES **iv**

ACKNOWLEDGEMENTS **vi**

ABSTRACT **vii**

CHAPTER 1: Introduction

1.1 Introduction 1

1.2 Definition of animal welfare 1

1.3 Measurement of animal welfare 4

1.3.1 Subjective feelings 5

1.3.2 Natural behaviours 7

1.3.3 Biological functioning 9

1.3.4 An integrative approach 10

1.4 Stress and its relationship to welfare 11

1.4.1 Definition of stress 11

1.4.2 Stress response 12

1.4.3 Distress and eustress 15

1.4.4 Acute and chronic stressors 16

1.4.5 Social stressors 17

1.4.6 Environmental stressors 17

1.4.7 Inter-individual variability 18

1.4.8 Measurement of the stress response 19

1.4.9 Factors effecting glucocorticoid levels 21

1.4.10 Glucocorticoids as a measure of stress 22

1.5 Animal welfare in zoological parks 24

1.5.1 Evaluation of welfare in zoological parks 27

1.6 Primates in zoological parks 29

1.6.1 Spider monkeys 30

1.7 Thesis outline 31

CHAPTER 2: Methods – Subjects and validation

2.1 Subjects and housing 34
2.1.1 Classification 34
2.1.2 Distribution 35
2.1.3 Conservation status 37
2.1.4 Spider monkeys at Chester Zoo 38
2.1.5 Enclosure details 42
2.1.6 Husbandry 42
2.2 General methods for urine collection 45
2.3 Assay validation 47
2.3.1 Aims 48
2.3.2 Methods 48
2.3.2.1 Immunological validation method 51
2.3.2.2 Immunological validation results 52
2.3.2.3 Biological validation method 53
2.3.2.4 Biological validation results 54
2.3.2.5 Creatinine assay 54
2.3.3 Summary 56

CHAPTER 3: Evidence that zoo visitors influence hypothalamic pituitary adrenal activity in spider monkeys (*Ateles geoffroyi rufiventris*)

3.1 Introduction 58
3.1.1 Factors effecting welfare of zoo housed animals 58
3.1.2 Zoo visitor studies 59
3.1.3 Factors influencing the impact of visitors on zoo primates 60
3.1.4 Using HPA activity to assess visitor impact 60
3.2 Aim 61
3.3 Method 62
3.3.1 Subjects and housing 62
3.3.2 Procedure 62
3.3.2.1 Urine collection 62
3.3.2.2 Quantification of levels of cortisol 62
3.3.2.3 Visitor study 63
3.3.3 Data analysis 64

3.4 Results 65
3.5 Discussion 66

CHAPTER 4: Patterns of injury in zoo-housed spider monkeys: A problem with males?

4.1 Introduction 70
4.2 Methods 72
4.2.1 Procedure 72
4.2.2 Analyses 73
4.3 Results 73
4.3.1 Frequency of aggression 74
4.3.2 Actors and intensity of aggression 75
4.3.3 Targets and intensity of aggression 79
4.3.4 Animal density 80
4.3.5 Context of aggression 80
4.4 Discussion 80

CHAPTER 5: The impact of social events on the hypothalamic pituitary adrenal axis of zoo-housed spider monkeys (*Ateles geoffroyi rufiventris*)

5.1 Introduction 87
5.2 Environmental factors influencing the stress response 88
5.3 Social factors influencing the stress response 90
5.3.1 Social buffering 91
5.3.2 Aggression 92
5.3.2.1 Dominance 92
5.3.2.2 Aggression within and between the sexes 93
5.3.3 Reproductive behaviour 94
5.3.4 Group formations and introductions 94
5.4 Spider monkey social dynamics 96
5.4.1 Spider monkey social organisation: its relevance to stressors in captivity 96
5.4.2 The dynamics of aggression in spider monkeys 96
5.4.3 The social dynamics of reproductive behaviour 98

5.4.4 Impact of social events in a zoo on HPA activity in spider monkeys 99
5.5 Aim 100
5.5.1 Predictions 100
5.6 Methods
5.6.1 Urine samples 101
5.6.2 Event categories 102
5.6.3 Defrosted samples 103
5.6.4 Analyses 104
5.7 Results 104
5.7.1 Aggression 105
5.7.2 Reproduction 110
5.7.3 Separation 113
5.8 Discussion 116

CHAPTER 6: Introduction of a new male to an established group of zoo-housed spider monkeys – A case study.

6.1 Introduction 123
6.2 Factors influencing introductions 125
6.3 Spider monkeys 126
6.4 Aim 127
6.5 Methods
6.5.1 Subjects 127
6.5.2 Procedure
6.5.2.1 Urine collection 127
6.5.2.2 Observations 128
6.5.3 Analyses 129
6.6 Results
6.6.1 Social behaviours 131
6.6.2 Self directed behaviour 135
6.6.3 Individual behaviour 136
6.6.4 Cortisol 137
6.7 Discussion 137

CHAPTER 7: Discussion

7.1	Thesis aims	144
7.2	The use of urinary cortisol to assess the stress response	144
7.3	Environmental stressors	145
7.4	Social stressors	146
7.5	Assessing the welfare in zoo-housed spider monkeys	149
7.6	Implications for other zoo studies	150
7.7	Further studies	151
7.8	Recommendations for management of zoo-housed spider monkeys	152

References

Appendices

A	Template for cortisol assay
B	Template for creatinine assay
C	Questionnaire
D	Behaviour check sheet for introduction of new male
E	Davis, N., Schaffner, C.M. & Smith, T.E. (2005). Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science* 90(2), 1331-141.
F	Davis, N., Schaffner, C.M., & Wehnelt, S. (2009). Patterns of injury in zoo-housed spider monkeys: A problem with males? *Applied Animal Behaviour Science* 116(2-4), 250-259.
G	List of conference presentations

TABLES

Table 1.1	Farm animal welfare council's Five Freedoms (FAWC 1992)	3
Table 2.1	Recognised species and sub species of *Ateles* including their latest conservation status	39
Table 2.2	Individual spider monkeys that served as subjects throughout the study period	41
Table 2.3	Summary of group demographics over the eight year study period	42
Table 2.4	Number of samples collected from each individual and for each corresponding study	46
Table 2.5	Information on the pools used in cortisol EIA for the different studies	51
Table 2.6	Number of samples used for each time block for the circadian rhythm validation	54
Table 3.1	Details of spider monkeys present during the visitor impact study	63
Table 4.1	Composition of groups by species/subspecies for group size, number of adults and non-adults, modal number of males and females and total number of aggressive incidents	75

Table 4.2	Summary of response to the questionnaire showing number of responses for each question and proportion of answers	76
Table 4.3	Summary of reported aggressive incidents towards adult male and non-adult male spider monkeys as classified by age, sex and intensity	77
Table 4.4	Summary of reported aggressive incidents towards adult female and non-adult female spider monkeys as classified by age, sex and intensity	78
Table 5.1	The number of urine samples used from each individual for each event	102
Table 5.2	LMM results for the three event categories	106
Table 5.3	Number of events and number of samples used for each type of aggression category	107
Table 5.4	Mean values and number of samples for every individual in the bystander role for each aggression and time category.	108
Table 5.5	Number of events and number of samples used for each type of reproduction category	110
Table 5.6	Mean values and number of samples for every individual in the other adult females role for each type of reproduction and time category.	111
Table 5.7	Number of events and number of samples used for each type of separation category	113

Table 5.8	Mean values and number of samples for every individual in each type of separation and time category	114
Table 6.1	Subjects of the experiment	128
Table 6.2	Ethogram	130
Table 6.3	Number of observations during each phase of the study	131
Table 6.4	Mean proportions of observation time for female resident behaviours that did not change significantly over the study period	133
Table 6.5	Changes in the mean proportions of observation time for new male's behaviour over the study period and for Ric in the Pre introduction phase	134
Table 6.6	Mean values of urinary cortisol for the previous male (Ric) and new male (Poy) over the study period	138

FIGURES

Figure 1.1	A model of biological response of animals to stress illustrating the three stages an individual experiences as a result of stress	14
Figure 2.1	Distribution of *Ateles* across Central America	36
Figure 2.2	Distribution of *Ateles* across South America	36
Figure 2.3	IUCN categories of threatened species	37
Figure 2.4	Photo of *Ateles geoffroyi rufiventris* at Chester Zoo	40
Figure 2.5	Photograph of the indoor spider monkey enclosure at Chester Zoo	43
Figure 2.6	Plan of the general arrangement of the indoor enclosure at Chester Zoo	43
Figure 2.7	Photograph of the spider monkey outdoor enclosure area at Chester Zoo	44
Figure 2.8	Plan of the general arrangement of the outdoor enclosure area at Chester Zoo	44
Figure 2.9	The binding ratio (%B/Bo) of two serial dilutions of the spider monkey urine pool A and the cortisol standards to demonstrate parallelism	53
Figure 2.10	Mean ± SEM levels of urinary cortisol across the time of day	55

Figure 3.1	Mean values ± SEM levels of cortisol for the visitor categories of no visitors; low visitors; medium visitors and high visitors	65
Figure 3.2	Urinary cortisol positively correlated with the number of visitors	66
Figure 5.1	% B/Bo of serial dilutions of the non defrosted and defrosted pools and cortisol standards to demonstrate parallelism	105
Figure 5.2	Mean values ± SEM levels of cortisol over time for the actor, target and bystander for the three aggressive events of A) minor; B) severe and C) lethal aggressive events	109
Figure 5.3	Mean values ± SEM levels of cortisol over time for the female, male and other adult females for the three reproduction events of A) ovulation; B) matings and C) birth	112
Figure 5.4	Mean values ± SEM levels of cortisol over time for all individuals for the three separation events of A) temporary; B) separation and C) reintroduction	115
Figure 6.1	Mean proportion ± SEM of observation time for female resident spider monkeys A) in no contact and B) in contact	132
Figure 6.2	Mean proportion ± SEM of observation time in female resident monkeys for groom other.	135
Figure 6.3	Mean proportion ± SEM levels of observation time in female resident monkeys for self-scratching.	136
Figure 6.4	Mean values ± SEM levels of urinary cortisol over time for female residents.	137

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ABSTRACT

The aim of this thesis was to provide a better understanding of the needs of spider monkeys (genus: *Ateles*) kept in zoological parks in order to provide an appropriate environment, which enhances the physical and emotional wellbeing of the individuals. This series of studies adopted primarily a physiological approach that entailed measuring cortisol in urine samples collected over a seven year period to assess the impact of a variety of social and environmental conditions. My studies also involved behavioural observations and a questionnaire study to collect information from other zoological parks that maintain groups of spider monkeys. In order to address the aims of my research I first validated an enzyme immunoassay for urinary cortisol which allowed for the activity of the HPA axis to be measured to assess the physiological stress responses in spider monkeys. The first study assessed the impact of visitors on spider monkeys by comparing levels of urinary cortisol collected with visitor numbers and I found an increase in visitor numbers was associated with an increase in cortisol. This was the first time the physiological impact of visitors was investigated and supports behavioural research that visitors adversely impact on primates in zoos. The second study I carried out involved a questionnaire to investigate frequency, direction and intensity of aggression in zoo-housed spider monkeys in 55 other zoos around the world. The pattern of aggression reported indicated severe and lethal aggression was relatively frequent among captive spider monkeys. Adult males were the most frequent actors of aggression and sub adult males were the most frequent targets, contradicting reports from wild spider monkeys. This aggression could be a condition of the management of spider monkeys in the zoos whereby males are normally transferred between zoos contradicting reports from the wild spider monkeys in which females would emigrate on reaching maturity. Next I investigated aggressive, reproductive and separation stressors on the spider monkeys housed at Chester Zoo over a seven year period and measured their effects via changes in urinary cortisol prior to, at and following each event. Aggression had the largest effect, with targets and bystanders having the highest levels of cortisol on the day of aggression for severe and lethal aggression, respectively. When examining the reproductive events, cortisol levels were significantly elevated in the mother the week prior to and the day of birth, but were

highest for bystander females on the day of birth. In the case of separations, cortisol was elevated when an individual was separated for longer than 24 hours for separations and less than 24 hour for reintroductions. Finally I investigated the replacement of the breeding male in the spider monkeys at Chester Zoo. Although a significant behavioural effect was identified in the adult females, there was little evidence of an increase in urinary cortisol among them. In addition, there were no instances of aggression between the adult male and juvenile male in the group.

Overall conclusions from this study indicate that the group of spider monkeys did demonstrate a varying stress response to a variety of social and environmental stressors associated with elevated cortisol levels and behavioural changes. However, there was no evidence of long term chronic stressors which are normally associated with poor welfare. This indicated that the environment provided for this particular group of zoo-housed spider monkeys generally allowed for the individuals within the group to cope and adapt. In light of these findings the study also makes a number of recommendations regarding the enclosure design, relocation of individuals and the gradual introduction of spider monkeys in zoos.

The findings of this study are important as it contributes to our understanding of the physiological responses to stressors in a zoo environment and therefore has implications for animal management. It also identifies potential species specific requirements for the spider monkey that should be considered.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Eliciting naturalistic behaviours from captive species has important consequences for education, conservation and scientific research (Carlstead, 1996; Mench & Kreger, 1996; WAZA, 2005). For the effective management of exotic animals in zoos the behavioural ecology of the species must be considered in order that an appropriate environment, which enhances the physical and emotional wellbeing of the individuals, can be provided (Carlstead, 1996; Robinson, 1998). Not only are zoological parks responsible for the provision of adequate food, shelter and health care, they must also provide the animals with the opportunity to express normal, natural behaviour within appropriate social settings.

To derive a full understanding of animal welfare researchers have investigated a variety of biological and social processes and their interplay. These include evolutionary history, behavioural ecology and proximate measures that cover behavioural and physiological events (Dawkins, 1980). Various factors have been documented that influence the physical and emotional wellbeing of animals (Dawkins, 1990). These include aspects of an individual's social environment (Gust, Gordon, & Hambright, 1993) and to a lesser extent their physical environment (Crockett, et al., 1995; Crockett, Shimoji, & Bowden, 2000).

1.2 Definition of animal welfare

Due to the subjective nature of animal welfare there have been doubts about the scientific validity of its measurement and assessment. The term did not originate as a scientific concept, but arose in response to the need to express ethical concerns regarding treatment of animals by humankind (Duncan & Fraser, 1997). It refers to an animal's quality of life (Broom, 2007) and involves making value judgements regarding elements such as physical health, 'happiness', and longevity for which people attach different levels of importance (Tannenbaun, 1991). However, due to

the importance of animal welfare and to allow for the formation of effective legislation, scientific research has been used in an attempt to evaluate and measure it (D. Fraser & Duncan, 1998).

Although it is generally accepted that welfare refers to a satisfactory or positive state, despite much discussion there is no universal, specific definition of it within scientific literature (Wiepkema & Koolhaas, 1993). Instead there are a variety of different interpretations from different researchers (Appleby, 1999). For example, Broom (1999) suggested a clearly defined concept of welfare is needed for use in precise scientific measurements, legislation and public discussion. It would then allow animal welfare to be compared across different situations or evaluated in a specific situation and assessed objectively. Attempts to define welfare as a purely scientific concept, however, have been questioned. D. Fraser (1995) argued that many of the proposed definitions have serious limitations. They specify very little about what processes contribute to overall welfare or how welfare may be measured. Instead D. Fraser (1995) sees welfare as a concept that involves subjective values on what is best for the quality of life of animals. Whilst scientific study can measure the various elements that are relevant to welfare, there is no objective method to combine them into a measure of the 'overall' welfare of the animal and can do little more than establish a general area of discussion (Duncan & Fraser, 1997; D. Fraser, 1995).

Animal welfare also has been defined as the state of an animal with regards to its attempt to cope with its environment (Broom, 1986), where coping refers to an animal's responses to help control its interactions with its environment and maintain mental and bodily stability; failure to cope means a reduction in fitness (Broom, 1991). This definition has since been clarified with welfare as a characteristic of an individual at a particular moment in time or over a longer period of time, and the term environment takes account of both internal and external stimuli (Broom, 2007). This definition recognises that welfare can be very good or very bad, and can vary on a continuum between the two extremes. It also implies good welfare is more than just the absence of disease or discomfort and emotional distress. It involves satisfying the animals' basic needs as identified by the Five Freedoms (FAWC, 1992).

One of the most widely used concepts of animal welfare encompasses the 'Five Freedoms of Animal Welfare' (see Table 1.1). This approach was first adopted

by the Farm Animal Welfare Council (FAWC), which was a body set up in response to the Brambell Committee (1965) to look at improving agricultural standards and practices in the UK. Much of the animal welfare legislation in the United Kingdom (e.g. Animal Welfare Act 2006, p. 7) is based upon these five freedoms.

Table 1.1 *Farm animal welfare council's Five Freedoms of Animal Welfare FAWC (1992).*

Freedom	Definition
1. Freedom from hunger and thirst	By ready access to fresh water and a diet to maintain full health and vigour
2. Freedom from discomfort	By providing an appropriate environment including shelter and a comfortable resting area
3. Freedom from pain, injury or disease	By prevention or rapid diagnosis and treatment
4. Freedom to express normal behaviour	By providing sufficient space, proper facilities and company of the animal's own kind
5. Freedom from fear and distress	By ensuring conditions and treatment which avoid mental suffering

The definition of animal welfare as adopted by the International Primatological Society (IPS) in their captive care guidelines goes beyond that captured by the Five Freedoms. It incorporates aspects of psychological wellbeing, the importance of social contact and acknowledges that for primates, the need to consider cognitive capabilities is also important. This latter definition is developed from the version used by the Association for Zoo and Aquariums (AZA) Animal Welfare Committee (McCann, et al., 2007).

> Animal welfare is the degree to which an animal can cope with challenges in its environment as determined by a combination of measures of health (including

pre-clinical physiological responses) and measures of psychological wellbeing. Good health represents the absence of diseases or physical/physiological conditions that result (directly or indirectly) from inadequate nutrition, exercise, social groupings, or other environmental conditions to which an animal fails to cope successfully.

Psychological wellbeing is dependent on there being the opportunity for animals to perform strongly motivated, species-appropriate behaviours, especially those that arise in response to aversive stimuli. Enhanced psychological wellbeing is conditional on the choices animals have to respond appropriately to variable environmental conditions, physiological states, developmental stages and social situations, and the extent to which they can develop and use their cognitive abilities through these responses. (p. 49)

1.3 Measurement of animal welfare

In order to meet the wide ranging definitions of welfare as captured by the Five Freedoms and the IPS guidelines, is it essential to be able to operationalise and measure welfare, although this also poses challenges. The measurement and assessment of welfare is a multidimensional discipline with a number of behavioural, physiological and biochemical techniques in use (Botreau, Veissier, Butterworth, Bracke, & Keeling, 2007; Broom, 2007; Dawkins, 2004; D. Fraser, 1995; Lane, 2006; G. Mason & Mendl, 1993). In addition, assessment can occur at different levels including the individual, the group or an entire husbandry system.

The method adopted by scientists, however, will depend on their adopted definition of animal welfare and their conceptualisation of it. Poor welfare has been indicated by a suppressed immune function (Honess, Marin, Brown, & Wolfensohn, 2005), gastric ulceration and anorexia (Bassett & Buchanan-Smith, 2007), reduced reproductive output (A. F. Fraser & Broom, 1990), aberrant behaviour (Dawkins, 2004; Wechsler, 2007), altered heart rate (Aureli, Preston, & de Waal, 1999; Clarke, Mason, & Mendoza, 1994; Honess & Marin, 2006), display of abnormal behaviours such as apathy and stereotypy (G. Mason & Latham, 2004) and increased activity in the pituitary-adrenocortical system (G. Mason & Mendl, 1993; Morgan & Tromborg, 2007; Wiepkema & Koolhaas, 1993). Other new areas of research include assessing

emotions and cognition (Boissy, Arnould, et al., 2007) including cognitive bias (Matheson, Asher, & Bateson, 2008) and brain measures (Broom & Zanella, 2004) as an indication of welfare.

Three approaches have been used systematically to investigate animal welfare (Appleby, 1999; Duncan & Fraser, 1997; Webster, 2005b). Each approach has a separate definition for animal welfare and consequently unique research strategies. The first approach is concerned with the animal's subjective feelings (Wemelsfelder, 2007). The second approach focuses on the extent to which animals display natural behaviours (Wechsler, 2007) and the third approach examines the degree to which an animal exhibits 'normal' biological functioning (Hughes & Curtis, 1997).

1.3.1 Subjective feelings

The first approach recognises animals as sentient beings and emphasises the subjective feelings of animals and the use of science to understand them (Wemelsfelder, 2007). It implies that animals have emotional capacities and will attempt to minimise negative emotions such as fear and frustration, while seeking positive emotions such as joy and pleasure (Boissy, Arnould, et al., 2007; Dawkins, 2006). The idea of an evolutionary continuity of animals experiencing emotions, such as fear or anger, is not new and was first proposed by Darwin (1871, 1872). However, whether animals experience even the basic range of states of consciousness, such as emotions, considering previous experiences or feeling pain, is difficult to assess and has been referred to as the 'hard problem' (Chalmers, 1995).

Although once seen as unscientific the idea of subjective feelings and emotions for animals is now generally accepted (Boissy, Arnould, et al., 2007), although there are still exceptions (Kennedy, 1992). The supporters of this approach argue that animal welfare is only affected if the animal is experiencing an unpleasant mental state, such as anxiety, boredom or frustration (Dawkins, 1990; Duncan, 1993; Brian O. Hughes, 1989; Sandøe & Simonsen, 1992). Therefore, even if an animal has health issues, or if its physical needs are not met, if the animal cannot feel these then there is no impact on its welfare.

Developing an understanding of unobservable processes involves additional logical steps and assumptions that are open to interpretation (Duncan & Fraser, 1997). It has been proposed that evidence of animal cognition and emotion could be

assessed through behaviour, by 'asking' the animals what they want through the use of preference tests, parallels with our own emotions and even brain imaging to investigate whether animals are sentient (Dawkins, 2006). The assumption is that the animals will make a valid preference that will either provide an increased positive or reduced negative state. However, there are limitations to this approach (D. Fraser & Matthews, 1997). An individual's choice could be affected by various factors such as individual differences, age, and experience, time of day or reproductive state, which potentially confounds such research. The link between preference and welfare is also limited to choices that are within an animal's capacity to make a valid choice; therefore it must fall within an animal's sensory or cognitive abilities. The concept of emotions however only refers to the immediate state of welfare of the animal at that time, and does not account for longer term issues or their general fitness (Webster, 2005b).

The use of cognitive science has also been highlighted as a means of researching emotions in animals as a means of improving their quality of life (Boissy, Arnould, et al., 2007). Another approach may be to use qualitative judgements of an animal's behaviour to assess their emotive state (Wemelsfelder, 2007). Providing it is based on knowledge of species-specific behaviours, and that an experienced person is making the assessment and animals are viewed as sentient beings, an approach that relies on subjective feelings can function in a scientific context.

Another method relies on the 'argument by analogy' by measuring responses to known unpleasant experiences in humans and looking for similar responses in animals. This relies on the presumption that mental suffering in animals is accompanied by similar physiological and behavioural responses to suffering in humans (Dawkins, 1990; Sandøe & Simonsen, 1992). The effect of emotional states on physiology offers opportunities for interpretation of subjective feelings in animals (Boissy, Manteuffel, et al., 2007; Gonyou, 1993). For example, the effect of emotions on humans influences the hypothalamic pituitary adrenal (HPA) axis and cortisol levels (Hodges, Jones, & Stockham, 1962). Studies in human participants have also shown a relationship between higher cortisol responses and greater expressions to negative emotions (Lewis & Ramsay, 2002, 2005). In animals further research is required to establish how such physiological changes in different species of animals can relate to various emotions (Désiré, Boissy, & Veissier, 2002).

1.3.2 Natural behaviours

The second approach to measuring welfare looks at providing natural environments that allow animals to perform most types of species-specific behaviour (Kiley-Worthington, 1989; Rollin, 1993; Shepherdson, 1999; Wechsler, 2007). The degree to which animals perform their natural behaviours is used as an indication of welfare state. The assumption being that the more 'natural' the behavioural repertoire displayed, then the better the animals' state of welfare. This idea is included in various animal welfare legislation and codes in the UK, which states that animals have a need to express most of their normal patterns of behaviour (FAWC, 1992; Thorpe, 1967). However, as animals have such behavioural diversity the consequences of not performing such behaviours will also be varied, particularly if the endpoint of a behavioural need is already provided in their environment (Baxter, 1983; Dawkins, 1983). For example, is there a behavioural need for anti predatory behaviour in captivity even when there are no predators present? Suffering should only result if an animal is highly motivated to perform a particular behaviour, but due to its environment becomes frustrated if it is unable to carry it out (B.O. Hughes & Duncan, 1988; Young, 1999).

Information regarding the species-typical behaviour patterns of wild animals (Stolba & Wood-Gush, 1989) can be used to establish normal behaviour patterns, and these ethograms could then be used to identify the specific behaviours that are important for the animals to perform (Veasey, Waran, & Young, 1996a). Motivation to perform these behaviours, such as foraging for food, could lead to suffering if they are not allowed, and the freedom to express normal behaviour is included in the UK animal welfare codes (MAFF, 1983). However, animals in the wild are regularly exposed to adverse conditions, not present in captivity and that are detrimental to their welfare, such as cold, hunger or the presence of predators (Veasey, Waran, & Young, 1996b). Therefore, a full repertoire of behaviour, which includes those evolved to cope with adverse conditions, could require the animals to be exposed to conditions that reduce welfare, which in turn have a negative impact on health (Dawkins, 2004).

An appreciation of the need for animals to express a greater range of species-specific behavioural repertoires has occurred more recently in the farming and zoo communities. For example, there has been a recent growth of interest in high welfare standard certification schemes in farming through both legislation and as a result of

consumer demand (Botreau, Veissier, Butterworth, Bracke, & Keeling, 2007; Broom, 2007). Modern zoological parks have also recognised the benefits of housing animals in a manner that encourages greater naturalistic behaviour by recreating more natural environments (Maple & Finlay, 1989). However, it is the outcome of the environment and its effect on promoting natural behaviour that is relevant to an animal's welfare rather than the natural habitat itself (Duncan & Fraser, 1997). In addition, this approach proves difficult to interpret into effective recommendations or detailed legislation (Appleby, 1999).

Contexts in the animals' captive environment that create internal uncertainty, such as absence of food or the presence of a rival can impact on an animal's welfare. Assuming the animal can resolve the internal conflict, such as they can find food or escape from a rival, then the response and impact on welfare is temporary (Wiepkema & Koolhaas, 1993) and may even mirror the challenges faced by wild-living individuals. Problems occur if the environment does not allow the animal to solve its internal conflicts, and the problem becomes chronic. Behaviours arising from normally transient internal conflicts can turn into disturbed behaviour, and become redirected to abnormal behaviour that is not normally part of an animal's natural behavioural repertoire. This indicates that the animal can no longer cope and its welfare is being seriously affected. These behaviours can then be used as behavioural indicators of poor welfare (Hughes & Duncan, 1988).

The behavioural indices of poor welfare have been separated into two categories. The first is quantitatively abnormal, whereby a behaviour is performed at a frequency or duration outside the range expressed in the wild, and would include hyperactivity, lethargy, social withdrawal, excessive aggression, over grooming and hyper-aggressiveness (Stevenson, 1983). The second is qualitatively abnormal, which includes behaviour patterns normally only expressed in captivity, and would include atypical postural movements, self mutilation (Hosey & Skyner, 2007), self clasping, cannibalism, sexual disorders, coprophagia, vomiting, eating of vomitus (Kuhar, 2008) and stereotypic movements (Stevenson, 1983). A specific type of abnormal behaviour in which the behaviour is repetitive and non-functioning is termed stereotypy (G. Mason, 1991). The presence of one or more stereotypies is a useful indicator of a sub-optimal or recently sub-optimal environment and considered as an indicator of poor welfare (Hughes & Duncan, 1988; G. Mason, 1991) although caution should be taken when using this in itself as an indicator of welfare. When

other welfare indicators are considered stereotypies can in some instances, particularly in sub optimal environments, be associated with an improvement in welfare (G. Mason & Latham, 2004). There is even evidence of stereotypies in some farmed animals being associated with a reduction in cortisol levels (Redbo, 1993; Vestergaard, Skadhauge, & Lawson, 1997).

1.3.3 Biological functioning

The third approach associates animal welfare with the physical and mental wellbeing or biological functioning of animals. Under this approach welfare is reduced by disease, injury and malnutrition (Hughes & Curtis, 1997) and improved welfare is indicated by high levels of growth and reproduction, normal physiological and behavioural processes, and ultimately high rates of longevity and biological fitness (Barnett & Hemsworth, 1990; Duncan & Fraser, 1997). The loss of body weight is also indicative of bad welfare, although for some species this may be a part of an animals' natural history, e.g. male cotton top tamarins (*Saguinus oedipus*) lose 10% of their body weight when they share in the carrying of young infants during the first 8 weeks of life (Achenbach & Snowdon, 2002). The biological functioning approach recognises that the welfare of the animal depends on its ability to cope with the response to incoming stimuli from the environment. Failure to cope will lead to a reduction in fitness, either by a reduction in life expectancy, a reduction in the number of offspring or an increase in inter-birth intervals. Furthermore, this approach refers to the state of an individual on a continuum from suffering to pleasure at any particular time throughout the animal's life. It is easier to scientifically demonstrate changes in biological functioning, although it can be difficult to interpret conflicting measures (Duncan & Fraser, 1997). A range of physiological and biochemical indicators have been used, including heart and respiratory rate, the endocrine system and immunity (Moberg, 2000).

However, physiological indicators can also vary during routine biological functioning, thus it can be difficult to determine welfare based on these measurements alone (Appleby, 1999). The concept of stress has been influential in this field with physiological studies into the endocrine system, and in particular the HPA axis and immune system (Lane, 2006; Mormede, et al., 2007; Veissier & Boissy, 2007). Behavioural assessments can also be useful in the interpretation of

levels of glucocorticosteroid hormones, which not only react to levels of stress, but can also be affected by physical activities such as locomotion and copulation (Toates, 1995). Validation of the stress response can also be attained by presenting an animal with a known stressor to ascertain its species-typical stress response and monitoring whether those same responses occur in other contexts (T. E. Smith, McGreer-Whitworth, & French, 1998).

It is also possible to correlate measurable physiological or behavioural changes with subjective feelings. This can be done by subjecting an animal to a known stressor and then measuring physiological and behavioural changes. If in another context similar responses occur it could be inferred that the animal finds it correspondingly unpleasant (T. E. Smith, et al., 1998).

1.3.4 An integrative approach

While all three approaches to animal welfare have their merits, in isolation they have limitations (Dawkins, 2004). The three approaches also do not always give the same conclusion. When this is the case, more information may lead to a consensus of opinion. However, the conclusion that is drawn will still be open to interpretation because it will depend on how much importance is attached to the different approaches in evaluating the welfare of the animal. One way to increase the objectivity to the three primary approaches in measuring animal welfare is to examine other potential indicators of sub optimal welfare.

While there are numerous methods available to measure particular aspects of animal welfare (Broom, 2007) and a great deal of progress has been made in this area over recent years, a checklist approach analysing a whole raft of physiological and psychological factors should be discouraged (Dawkins, 2004). There are difficulties in interpreting the results, as well as practical, financial and temporal constraints (D. Fraser, 1995; Lane, 2006). Changes in various indicators may result from unpleasant subjective feelings, but they cannot be directly measured, and do not necessarily provide evidence of suffering. There could be limitations to such a global approach as responses may be dependent on a specific situation, or may only be relevant as short-term or long-term indicators. Furthermore, different measures of assessment may provide contradictory results with repeated assessments giving differing responses (G. Mason & Mendl, 1993).

An important consideration for animal welfare is the ability for an individual to have control over its environment, with poorer welfare if the animal does not have control (Bassett & Buchanan-Smith, 2007). Individual animals also give a high priority to the behaviours that promote and maintain their life conditions (Wiepkema & Koolhaas, 1992). Such behaviours imply learning to predict or control their position within their environment and if there are problems with these processes it could have welfare implications (Wiepkema & Koolhaas, 1993). Many relevant temporal and spatial relationships in nature have a degree of variability. While these may evoke a degree of novelty or uncertainty and could even result in a short term reduction in welfare, their range is normally within the coping capabilities of the individual and would not cause lasting harm. The ability of the individual to cope with some short term reduction in welfare makes it difficult to assess what the acceptable limits of an individual are within a captive environment (D. Fraser & Duncan, 1998).

1.4 Stress and its relationship to welfare

The social and physical environment likely provides a constant source of physical and emotional challenges or stressors to an animal and the animal must respond to each challenge either to remove it, or to adapt to cope with it. This in itself is not necessarily bad and can even be beneficial if it remains within the ability of the individual to cope (Wiepkema & Koolhaas, 1993). However, if the stress levels remain beyond the abilities of the individual to cope or adapt either within the short- or long-term, then its welfare will be affected (Broom, 2007). Therefore, stress refers only to situations when something challenges an individuals mental or physical capacity to cope, and poor welfare refers to the state of the animal when the individual is having difficulty coping (Webster, 2005a). Stress has such a profound impact on animal welfare it is essential that an understanding of the biological response to stress is developed (Moberg, 2000).

1.4.1 Definition of stress

Like welfare, the term “stress” has been used broadly in biology and no clear universally accepted definition currently exists (Moberg, 2000). Indeed the word has

been used to describe such a wide range of symptoms and levels of suffering that it has even been argued that it has become an essentially useless term (McEwen, 2000). However, the widespread use of the term necessitates its continued use providing it is clearly defined (Morgan & Tromborg, 2007).

Broom (1999) defined stress as "an environmental effect on an individual which over-taxes its control systems and reduces its fitness or seems likely to do so" (p. 138). Stress has also been defined as a chronic disturbance of the processes that underlie adaptive behaviour, i.e. a long-term failure of regulation by systems that normally exhibit effective negative feedback (Toates, 1995). A similar view captures the definition as the experience of having internal or external demands that exceed an individual's resources for responding to those demands (Danzter, 1991). The word 'stress' should be used for the aspect of poor welfare which involves the failure of that individual to cope (Broom, 1999). Consequences of stress can lead to reduction in general health, which could manifest itself as a reduction in growth rate, and a reduction in the immune system and in turn greater susceptibility to disease.

Moberg (2000) defined stress as the biological response elicited when an individual perceives a threat to its homeostasis. The threat is the 'stressor' that can be anything which changes the homeostasis of the animal, although this is an oversimplification as it does not take into account the daily physiological adjustments required to adapt to a changing environment (Goymann & Wingfield, 2004; McEwen & Seeman, 1999). The term stress also refers to both the stimulus that provokes a response as well as the resulting internal changes induced by the stimuli. For clarity, it has been suggested that the word stress should be avoided, and replaced with the terms stressor (the stimuli) and stress response (the change in internal state) (Creel, 2001) and this has been adopted in this study.

1.4.2 Stress response

Hans Selye (1946) first identified the 'General Adaptation Syndrome' (GAS), to describe the common features of the physiological response of humans to a wide range of physical or physiological challenges he called 'stressors'. In summary he proposed that a body would respond in a non specific, systematic response on exposure to any type of stress. However, largely due to the work of Mason (1971), it became clear that different stressors can evoke their own specific stress responses

(behaviourally and physiologically), possibly resulting in the non-specific response, and that most stressors are characterised by an aspect of novelty that has a great psychological impact. More recently, the early view of a generalised non-specific stress response to all stressors, such as that initially proposed by Selye (1946), has been disproved (Wiepkema & Koolhaas, 1993).

Moberg (2000) identified three general stages in a stress response; recognition of a stressor; the biological defence to the stressor; and the consequences of the response. It is the last stage that will determine whether the animal is experiencing distress. A 'stressor' could be an actual physical challenge to homeostasis, such as a change in temperature, physical restraint, or a threat to a change, such as an approach of a human, or stare of a dominant individual. Whether the stimulus is actually a threat is not important, it is the perception of the threat that is important. This is why psychological stressors can be damaging (McEwen, 2000).

A model of stress, including recognition, responses to and the consequences of stress is presented in Figure 1.1. The first stage of the stress response is the recognition of the threat by the central nervous system (CNS). The body then develops a biological response which involves a cascade of physiological events designed to prepare the body for a threat to its homeostasis, the 'fight-flight' response (Cannon, 1929, as cited by Moberg, 2000). This second stage consists of a combination of the four general stress responses (the behavioural response, the autonomic nervous system response, the neuroendocrine response or the immune response).

The behavioural response is normally the first reaction by an animal with an attempt to avoid the stressor by simply removing itself from the threat (Sapolsky, 2000). This is followed by the autonomic nervous system which controls a diverse number of biological systems, including changes in neurotransmitter levels, changes in the cardiovascular system, shutting down the gastrointestinal system, activating the exocrine glands and the adrenal medulla. These have a variety of effects including tachycardia, increases in the rate of respiration and increased glucose metabolism. However, as such effects are very specific, difficult to measure and relatively short in duration the autonomic nervous system has been of only limited interest in the study of an animal's long term welfare (Moberg, 2000).

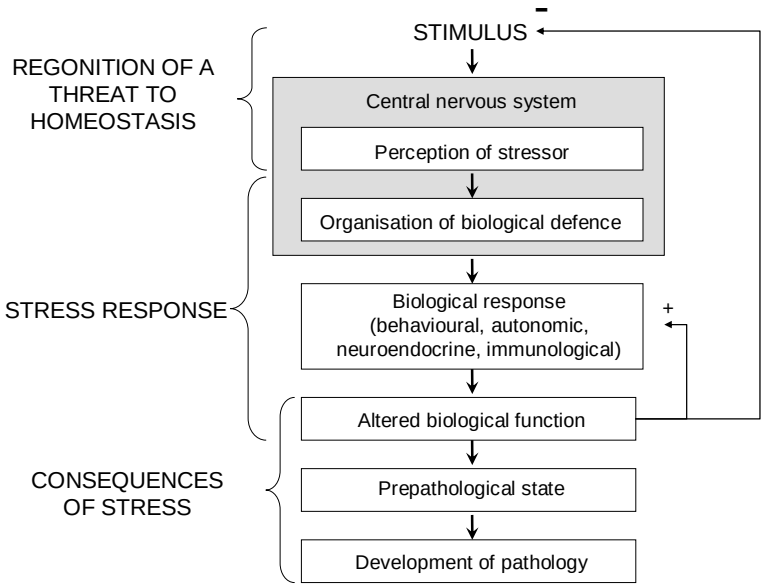

Figure 1.1 A model of biological response of animals to stress illustrating the three stages an individual experiences as a result of stress (modified from Moberg, 2000).

In contrast, the neuroendocrine system, which confers another biological response to stress, can have a broad and long lasting effect on the body. During periods of stress the HPA axis is stimulated, initially to produce corticotrophin releasing hormone (CRH) and vasopressin (AVP) from the hypothalamus. This in turn stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, which leads to the production of glucocorticoids (GCs) (cortisol and corticosterone) from the adrenal cortex (Mormede, et al., 2007). They are responsible for nearly all the biological functions affected by stress, such as changes in the immune system, reproduction, metabolism and behaviour (Matteri, Caroll, & Dyer, 2000). Other endocrinological responses to stress include the secretion of prolactin, growth hormone, thyroid stimulating hormone and gonadotrophins. Finally, the immune system also responds directly to a stress response, and although this has yet to be fully understood, it provides a potentially powerful alternative tool to evaluate an animal's response to stress (Honess, et al., 2005; Moberg, 2000).

The stress response can be considered adaptive, enabling animals to escape from or cope with a threat (Wiepkema & Koolhaas, 1993). Together, these biological

responses are essential for surviving the regular exposure of various stressors (Sapolsky, 2004). Providing there are sufficient biological reserves to deal with the cost then there are no biological consequences of the stress response. If there are insufficient reserves to deal with the biological cost then the consequences are that resources will be shifted away from essential biological functions, and the animal will be left in what has been referred to as a prepathological state.

1.4.3 Distress and eustress

Only when the stress response threatens the animal's wellbeing does it experience 'distress'. This term helps to differentiate between a non-threatening stress response and a biological state when the stressor starts having a negative impact on the individual's welfare (Moberg, 2000).

While care should be taken to avoid unnecessary stressors in animals kept and managed in a captive environment some forms of stress are unavoidable, although not necessarily detrimental (Chamove & Moodie, 1990). Indeed, an animal living within its natural environment is often exposed to a variety of serious and potentially life threatening stressors (Sapolsky, 2000). These include hunger, thirst, injury through conspecific aggression or attempted predation and a variety of social stressors (Veasey, et al., 1996b). Animals maintained by human beings, whether as a pet, in a farm, laboratory or a zoo setting, normally benefit from regular provision of food, water, shelter and veterinary aid, factors that can reduce stress.

Not all stressors that evoke a stress response are detrimental to an individual. A stress response can provide stimulation that is beneficial to the animal by optimising vigilance (Wiepkema & Koolhaas, 1993), facilitating the activation of reproduction (G. M. Barrett, Shimizu, Bardi, Asaba, & Mori, 2002; Engh, et al., 2006), enhancing learning, increasing alertness and exploration (Chamove & Anderson, 1989; Chamove & Moodie, 1990), and even improving immune responses in the short term (Ellard, Castle, & Mian, 2001). This 'good stress' may even be perceived as pleasurable, and as a concept has been described as 'eustress' (Selye, 1974). The difference between distress and eustress is biological cost. An animal has evolved to be able to cope with a short term stressor, such as an attack by a predator, providing the animal has enough reserve to cover the cost of the stressor (Sapolsky, 2000).

1.4.4 Acute and chronic stressors

Generally the effects of chronic stress are more likely to have an impact on an animal's welfare than acute stress (Lane, 2006). For example, chronic stress can make an animal more prone to infections due to suppression of the immune system, whereas an acute stressor actually enhances the immune function leading to a short term protection against disease (Lane, 2006).

Chronic stress is caused either by repeated exposure to the same stressor, or by simultaneous exposure to several active stressors, both of which must persist over a long period of time and whose accumulative biological cost initially forces the animal into sub clinical stress (Moberg, 2000). Under these conditions, although biological functions may not be affected, they make the animal more vulnerable to distress when exposed to a further stressor. Long-term chronic stress should be thought of as a series of repeated exposures to acute stressors, rather than a constant and unvarying condition (Ladewig, 2000). This has particular relevance for an animal that is kept in sub optimal conditions, such as inappropriate housing environments or social contexts leaving them more susceptible to another, normally innocuous stressor. Although long-term chronic stress is a more likely cause of distress, depending on its severity and timing, an acute stressor can also cause a major welfare problem (Lane, 2006; Moberg, 2000).

Acute stressors are short term and normally associated with an initial behavioural response of orientation, alarm and vigilance. Often they are easier to cope with as the animal may be successful in avoiding the stressor by simply removing itself from the threat (Moberg, 2000). Such a response will not be appropriate for all stressors or if the animal finds itself in a position where such behavioural options are restricted (Ladewig, 2000). Even when a behavioural response does not alleviate the stressor, a component of it may still be a part of the stress response and thus provide a potential clue to distress (Rushen, 2000). However, insufficient understanding of the behaviour of animals during stress limits the value of using behaviour as a means of predicting distress.

All animals in captivity should have their stress levels managed, just as their nutrition or reproduction is managed (Moberg 1992, 1993). The strategy should be to minimise the biological costs of stress at all times and never allow it to rise above subclinical levels.

1.4.5 Social stressors

While social groups provide the advantage of support and co-operation, there are also disadvantages of increased conflict and competition (Goymann & Wingfield, 2004; Kikusui, Winslow, & Mori, 2006). Research in non-human primates has shown that social stress is especially effective in producing chronic changes in the function of the HPA axis, although the effect is influenced by the species' social organisation and an individual's position within it (Engh, et al., 2006; Smith & French, 1997b; Ziegler, Scheffler, & Snowdon, 1995). For example, social instability, unnatural isolation, dominance, introductions and separations all demonstrate behavioural and physiological impacts on stress responses (Honess & Marin, 2006; Paker, Collins, Sindimwo, & Goodall, 1995; Sapolsky, 2005), although the responses differ widely across various species (Setchell, Smith, Wicking, & Knapp, 2008).

1.4.6 Environmental stressors

Animals have adapted a variety of behavioural and physiological responses to deal with the diverse challenges that natural surroundings can offer (Morgan & Tromborg, 2007). When animals are kept in captivity their ability to deal with stressors can be affected if they are not allowed to or are unable to carry out these responses. The lack of complexity, restricted movement, lack of retreat space, forced proximity to humans, routine husbandry and restricted foraging have all been identified as potential environmental stressors for captive animals (Morgan & Tromborg, 2007).

A variety of studies examined various environmental features and their impact on animals in captivity. For example, the provision of appropriate substrates stimulates more natural foraging behaviour patterns (Beisner & Isbell, 2008; Chamove, Anderson, Morgan-Jones, & Jones, 1982; Dawkins, 1983; Lutz & Novak, 1995). Studies assessing enclosure size (Clubb & Mason, 2007; Crockett, et al., 1995), environmental enrichment (Carlstead & Shepherdson, 2000; Schapiro, Bloomsmith, Kessel, & Shively, 1993), novelty (T. E. Smith, et al., 1998) and husbandry procedures and routines (Bassett & Buchanan-Smith, 2007; Line, Morgan, Markowitz, & Strong, 1989) have demonstrated that these also impact on the physiology and behaviour of captive animals. The prevention or interference by the

captive environment on an animal's ability to perform certain species-specific behaviours, for which animals may have a behavioural need, can also cause a stress response (Morgan & Tromborg, 2007).

Captive environments are generally less complex than natural habitats and consequently animals have a reduced amount of environmental control and an increased amount of predictability (Carlstead, 1996). It is this lack of control and variations in predictability that are potentially the greatest stressors for animals in captivity (Bassett & Buchanan-Smith, 2007; Sambrook & Buchanan-Smith, 1997; Wiepkema & Koolhaas, 1993). The importance of predictability and control has been demonstrated in a classic study on rats (*Rattus norvegicus*) (Weiss, 1972). When two rats were subjected to a series of identical electric shocks, but one was able to predict and control the shock by means of a warning light and the use of an adjustable wheel, its GC response was significantly reduced.

1.4.7 Inter-individual variability

Inter-individual variability to the stress response is well documented with different disease patterns resulting across animals experiencing the same stressor (Boccia, Laudenslager, & Reite, 1995; Moberg, 1985, 2000; Mormede, et al., 2007). This variation is consistent and stable over time, and a given stress response style may remain characteristic to an individual over its lifetime (Pottinger, 2000). A number of factors that may contribute to this individual variation have been highlighted (Mormede, et al., 2007). They include: past experiences (Kikusui, et al., 2006; W. A. Mason, 2000), age (Honess & Marin, 2006a), social status (McGlone, et al., 1993), genetics (Pottinger, 2000), reproductive state (Cavigelli & Pereira, 2000; Ziegler, Scheffler, & Snowdon, 1995), temperament (A. S. Clarke & Boinski, 1995; Maestripieri, 2000), rearing history, (Boccia, Laudenslager, & Reite, 1995; Dettling, Feldon, & Pryce, 2002) and even time of year (Carlstead & Seidensticker, 1991). While it may be possible to monitor individuals under carefully controlled laboratory conditions, even to the advantage of learning more about how the stress response is affected, it can be more difficult to know the past experience, social relationships or genetic predisposition to a stress response in a less controlled environment, such as a farm or a wild population (Mormede, et al., 2007).

Due to the high degree of individual variation to stressors it is better to use animals as their own control using repeated measures design (Honess & Marin, 2006a). As acute and chronic stress can both lead to negative effects on welfare the key to the use of GCs for assessment must lie in repeated sampling as a stand alone measure of GC can be misleading (Lane, 2006).

1.4.8 Measurement of the stress response

While there are a variety of physiological and behavioural indicators that can be used for measuring an individual's response to stress it is important to determine which methods are the most reliable, accurate and appropriate. It is therefore not expected that any particular indicator of stress will be appropriate for all types of stressors (Moberg, 2000). To complicate the matter further these systems can also often have similar responses to both harmful and innocuous stimuli.

Behavioural indices of stress are attractive as they are relatively easy, non invasive and inexpensive to obtain when compared to physiological measures. Behaviour has also been considered to more accurately reflect the animals underlying dispositional state than physiological measures (Dawkins, 2004, 2006). Displacement behaviours, which are activities that are characterised by their apparent irrelevance to ongoing activities (Tinbergen, 1952), can also be used as indicators of an individual animal's emotional state (Maestripieri, Schino, Aureli, & Troisi, 1992). In non human primates such behaviours include yawning, scratching, auto grooming and body shaking (O. N. Fraser, Stahl, & Aureli, 2008; Maestripieri, et al., 1992). The exhibition of these behaviours is consistently accompanied by physiological changes such as increases in heart rate, blood pressure and GCs, which are associated with a stress response. Pharmacological validation of displacement behaviour by using anxiety inducing and reducing drugs leads to corresponding increases and decreases in the rate of displacement behaviours, respectively (Barros, et al., 2007; Gabriele Schino, 1996). Further investigation is also required to associate the various behaviours and emotional states (Maestripieri, et al., 1992).

There are also a number of normally rare and distinctive behaviours whose presence is associated with extreme levels of stress in primates. These include self-mutilation and stereotypies and many studies have used them as indicators of high stress levels (Honess & Marin, 2006a). Another approach includes quantitative and

qualitative changes in the overall behaviour repertoire (Rushen, 2000). However, the control of behaviour in response to stress is complex. Until the underlying causal mechanisms of behaviour during stress are fully understood they are difficult to interpret as a means of identifying stress. There is also no general behavioural stress response shown by an animal during stress, rather the behavioural response is specific to the stressor (Rushen, 2000). In non-human primates there is also evidence that increased locomotion may be part of a generalized stress response in some species (T. E. Smith, et al., 1998).

More invasive procedures are often used within laboratory settings. For example, the monitoring of autonomic stress responses, such as changes in heart rate or blood pressure, has been used in non human primates (Boccia, et al., 1995; S. Line, K. Morgan, H. Markowitz, & S. Strong, 1989; Line, Morgan, Markowitz, & Strong, 1990). However, this requires the implantation of telemetry devices which may confound and compound measures of stress (Honess & Marin, 2006a). Another method which has been successfully used involves the monitoring of leukocyte activity, which is known to be affected by psychological stress in humans (Ellard, et al., 2001). This technique has also been adopted in badgers in the wild (Montes, McLaren, Macdonald, & Mian, 2004) and more recently in non human primates (Honess, et al., 2005). However, the procedure requires blood samples which involve separation, capture and restraint, all of which are known to cause a stress response (Mormede, et al., 2007).

Finally, the monitoring of the neuroendocrine system has also been successful in measuring the stress response, and the most widely studied and dependable index uses adrenocortical hormones (Kikusui, et al., 2006). There are a number of hormones involved in the HPA axis including CRH, AVP, ACTH and GCs (see section 1.4.2). These cholesterol-derived steroids are produced as cortisol in most mammals and fish, and as corticosterone in rats and birds (Mormede, et al., 2007). Although increased levels of GCs have been recorded in many species and in every vertebrate genus (S. L. Klein, 2000), there are large inter-specific differences in basal levels of cortisol. Such differences are however comparable for the majority of physiological and behavioural parameters (Lane, 2006). The measurement of these GCs following activation of the HPA axis is a well established means of assessing stress levels in animals (Buchanan & Goldsmith, 2004; Mendoza, Capitanio, &

Mason, 2000) and can provide important information which can help in assessing the welfare status of an individual or group of animals.

1.4.9 Factors effecting glucocorticoid levels

There are a number of factors that need to be considered when using GCs as a measure of stress (Honess & Marin, 2006; Lane, 2006; Millspaugh & Washburn, 2004) and these should ideally be controlled for in any research study. For example age, sex and reproductive status may influence the activity of the HPA axis (Millspaugh & Washburn, 2004), although the means by which it is influenced is not clearly understood (Tilbrook, Turner, & Clarke, 2000).

Although there is only limited evidence of any sex differences in basal GC levels (Lane, 2006; Tilbrook, et al., 2000), there is considerable evidence that there can be significant sex differences in responses to different types of stressors (Lane, 2006; Silva, Ines, Nour, Straub, & Da Silva, 2002). Cortisol levels in females are affected by physiological changes during ovulation, pregnancy and lactation. For example, cortisol levels in humans are approximately three times higher during pregnancy compared to non pregnant levels, rising to five times in late gestation (Keller-Wood & Wood, 2001). There are also well established links between reproductive status and basal cortisol levels with elevated cortisol levels during late gestation demonstrated in a variety of other primates (Cavigelli, 1999; T. E. Smith & French, 1997a; Weingrill, Gray, Barrett, & Henzi, 2004).

It has been suggested that exercise should be considered as a variable when considering GCs as a measure of stress (Coleman, et al., 1998). Cortisol has a role in metabolic homeostasis, in particular in the regulation of energy, thus any change in the HPA axis is not always necessarily a consequence of a stressful stimulus (Mormede, et al., 2007). For example, an increase in urinary cortisol levels has been found to be positively correlated with locomotion in some primates, and may be part of a generalised stress response in some species (T. E. Smith, et al., 1998). However, in other studies where this has been assessed, significant increases have only been found for extreme levels of exercise (Lane, 2006). These studies indicate that providing energy requirements can be met by existing fat and carbohydrate stores, for example during moderate or short intense periods of exercise, levels of GCs would not be affected.

Other factors that need to be considered when using GC levels include the natural circadian cycle of circulating cortisol, with GC levels known to be much higher in the morning than later in the day for diurnal animals (Mendoza, et al., 2000). Seasonality has also been reported to have an effect on GCs in a variety of animals, whereby levels vary at a predictable level throughout the annual cycle (Millspaugh & Washburn, 2004). This can be linked to seasonal changes in environmental conditions that can impact on the individual's metabolic demand (Lane, 2006), such as extremes of temperature and humidity (Weingrill, et al., 2004), reproductive status (Honess & Marin, 2006a) or food availability (Cavigelli, 1999). Nutritional status also affects GC levels, particularly for faecal cortisol measurements (Millspaugh & Washburn, 2004), although day-to-day changes in diet are not thought to have a significant impact (Lane, 2006). These external factors are particularly relevant to wild and free range studies whereby such conditions, unlike captive studies, are not under the researcher's control.

1.4.10 Glucocorticoids as a measure of stress

Glucocorticoid hormones can be measured in several biological samples, including plasma, saliva, urine and faeces. Plasma is the most widely used in animal welfare studies (Mormede, et al., 2007), and its benefits include providing an instantaneous value of GCs in the blood at the time of the sample. However, there are also a number of conflicting factors that need to be considered.

There is potentially a large variation in values depending on when the sample is taken in relation to when the stressor occurred (Lane, 2006). The HPA axis is highly sensitive and it only takes a few minutes following an event before an increase in GCs can be detected in the blood. The response is then prolonged for around one hour following the cessation of the event (Mormede, et al., 2007). The timing of the blood sample is therefore important with at least ten minutes required before the GCs can be picked up in the blood (Mormede, et al., 2007). This sensitivity also requires consideration for blood sampling procedures. Separation of individuals from their social partners, capture, handling, physical restraint and even anesthetisation are often required, and these events stimulate HPA activity (Schaffner & Smith, 2005; T. E. Smith & French, 1997a). Even animals that are trained to present a limb for samples have shown a stress response to the procedure (Honess &

Marin, 2006a). Such invasive procedures are likely to confound the interpretation of results. Therefore, where a hands-off study is not possible, the use of appropriate control animals is essential (C. J. Cook, Mellor, Harris, Ingram, & Matthews, 2000). While remote blood sampling through various devices has been offered as a solution (Ingram, Crockford, & Matthews, 1999), the development of non invasive techniques are favoured as they minimise the impact on the animal and allow the study of animals whilst in their 'natural' situation (Buchanan & Goldsmith, 2004).

The use of salivary GCs has been used to monitor stress response in a variety of non–human primate species including rhesus monkeys (*Macaca. mulatta*) (Boyce, Champoux, Suomi, & Gunnar, 1995), orang-utans (*Pongo pygmaeus*) (Elder & Menzel, 2001) and baboons (*Papio hamadryas*) (Pearson, Judge, & Reeder, 2008). A number of methods have been adopted to collect saliva including offering flavoured rope to be chewed (Lutz, Tiefenbacher, Jorgensen, Meyer, & Novak, 2000; Pearson, et al., 2008). It offers the advantage of being relatively non invasive, and offers a less stressful alternative to blood collection for measuring short-term stressors. However, there are some limiting factors that need consideration, with difficulties in its use in untrained and wild animals as there are problems with considerable inter-individual variation in the time lag from the bloodstream to saliva and the impact of circadian rhythms (Lane, 2006). There are also some sensitivity and specificity issues (Mormede, et al., 2007).

The analysis of faecal samples for measuring GCs has been successfully used to monitor stress responses in a variety of animals (Boinski, Swing, T.S., & Davis, 1999; Shepherdson, Carlstead, & Wielebnowski, 2004; Whitten, Stavisky, Aureli, & Russell, 1998). It also offers a non invasive means of collection, and is particularly appropriate in free range and field studies (Cavigelli, 1999; Engh, et al., 2006) making it a particularly useful tool in the field of conservation biology (Millspaugh & Washburn, 2004). Variability has been reported in faecal GC measurements, which may be due to potential dietary effects, water content, collection and storage techniques and assay protocols (Lane, 2006).

Urinary GCs provide another non invasive method, although the practical difficulties in collection make it more prominent in captive studies. It has been used to examine cortisol levels in marmosets (*Callithrix kuhlii*) (Schaffner & French, 2004; T. E. Smith & French, 1997a), brown capuchins (*Cebus apella*) (Boinski, Swing, Gross, & Davis, 1999), pig tailed macaques (*Macaca nemestrina*), (Crockett,

et al., 2000), chimpanzees (*Pan troglodytes*) (Whitten, Stavisky, Aureli, & Russell, 1998) and cotton topped tamarins (*Saguinus oedipus*) (Ziegler, et al., 1995). . Urine is also the main elimination pathway for GCs, therefore its measurement accounts for the accumulation of cortisol over several hours (Mormede, et al., 2007), although this is subject to species variation. The peak excretion in urinary cortisol in three species of primates was found to be around six hours post stressor (Bahr, Palme, Möhle, Hodges, & Heistermann, 2000). Urinary GCs have the benefit of adjusting for the fluctuations present in plasma levels, therefore providing an integrative, sensitive measure of their production over a period of time (Mormede, et al., 2007). However, such consistency could be seen as a disadvantage if more temporal precision is required (C. J. Cook, et al., 2000). It is also necessary for urinary cortisol levels to be corrected for diuresis by correcting for creatinine levels (Burtis & Ashwood, 2001).

1.5 Animal welfare in zoological parks

In recent years it has been recognised that zoos and wildlife parks have an important educational role in raising awareness of the environmental issues that threaten the survival of animal species in the 21st century (WAZA, 2005). For visitors to be connected or inspired to care about these issues it is important that the animals are maintained at the highest welfare standards and behave as naturally as possible (Kidd, Kidd, & Zasloff, 1995; Robinson, 1998). This is achieved by housing the animals in an appropriate naturalistic context, to encourage their 'natural' behaviour within the appropriate surroundings. In addition, any animals that show abnormal behaviours or appear to be suffering will be counter productive to the conservation message (Carlstead, 1996).

Negative or abnormal behaviours have been documented in zoos and include lethargy, inappropriate self-directed behaviours such as self rocking or self mutilation (Hosey & Skyner, 2007), coprophagy, excessive aggression and the performance of natural behaviours, but performed out of context or to an excessive manner (e.g. over grooming) (Carlstead, 1996). Stereotypies are also prevalent in zoos, with some species being particularly likely to display them (e.g. 82% of zoo carnivores or 47% of zoo or circus elephants (G. Mason & Latham, 2004).

The importance of natural behaviours in zoos was first highlighted by Hediger (1950). However, they were not used as a benchmark of animal welfare until much more recently (Chamove & Anderson, 1989; A. F. Fraser & Broom, 1990a; Lindburg, 1988; Thorpe, 1967). Furthermore, assessment and the presence of natural behaviours needs to be based on scientific evidence, and not on preconceptions, perceived behaviour or anthropomorphisms (Robinson, 1998). It must also be remembered that the wild environment can be a challenging place and does not necessarily provide a blueprint for optimal welfare (Veasey, et al., 1996b).

However, other behaviours can be stimulus driven, and in the absence of the stimulus there is no motivation (Veasey, et al., 1996b). One such example could be predatory avoidance behaviour, and in these cases providing there is no stimulus, the absence of such behaviour would not indicate a loss of welfare. Therefore, the performance of a full repertoire of natural behaviours is not necessarily essential for the welfare of an animal kept in captivity, i.e. an animal behaving differently to that of wild conspecifics is not necessarily suffering (Veasey, et al., 1996b). While some work has been done with domesticated animals regarding behavioural needs (Dawkins, 1990; Jensen & Toates, 1993), such as dust bathing in poultry (*Gallus gallus*) (Dawkins, 1983) and nest building in sows (*Sus scrofa*) (Jensen, 1993), surprisingly little work has been done with zoo-housed animals. Indeed for many of the species kept in zoological parks very little information is known about their behaviour in the wild (Robinson, 1998). This presents a challenge for zoo management and their staff to provide the appropriate environments for their animals.

Animals housed in artificial environments are exposed to a wide variety of potential stressors (Morgan & Tromborg, 2007). While zoological parks are not constrained to the same degree as laboratories and some farming paradigms, there are still unique challenges to housing animals within a zoo setting (Hosey, 2005; Robinson, 1998). These include the effects of visitors (Birke, 2002; S. Cook & Hosey, 1995; Hosey, 2000; Mitchell, et al., 1992), keeper animal interactions (Hosey, 2008; Mellen, 1991; Wielebnowski, Fletchall, Carlstead, Busso, & Brown, 2002), unpredictable noise (Owen, Swaisgood, Czekala, Steinman, & Linburg, 2004; Shepherdson, Carlstead, & Wielebnowski, 2004), construction work (Powell, Carlstead, Tarou, Brown, & Monfort, 2006), proximity to predators, prey or competing conspecifics (Buchanan-Smith, Anderson, & Ryan, 1993; A. Lee, 1992;

Wielebnowski, et al., 2002), imposed feeding regimes (Ames, 1993; Bassett & Buchanan-Smith, 2007; Bloomsmith & Lambeth, 1995), introductions to new environments and new group formations (Brent, Kessel, & Barrera, 1997; Doyle, Baker, & Cox, 2008; Soltis, Wegner, & Newman, 2003), regular veterinary procedures (Grandin, 2000; Laule, Bloomsmith, & Schapiro, 2003) and training (Bassett, Buchanan-Smith, McKinley, & Smith, 2003).

In practice it is still as difficult to assess the costs of captivity for animals as it is to assess its benefits to individuals, species and ecosystems (Mench & Kreger, 1996). In recent years there have been significant improvements in the care of animals in zoological parks, in particular in their physical comfort, nutrition and general health resulting in a great reduction in morbidity and mortality (Mench & Kreger, 1996). While it is fairly straightforward to meet the physical needs of most animals in zoological parks, there are concerns that they are still not meeting the more esoteric physiological or behavioural needs of a wide variety of rare and endangered animals (Mench & Kreger, 1996). It is also possible that some species requirements just cannot be met adequately in captivity and therefore are not suitable for life in captivity (Robinson, 1998).

Maintaining the behavioural diversity of a species, which includes a full repertoire of natural behaviours, has important conservational significance (Markowitz, 1997). Ex-situ breeding programmes in zoological parks have been successful in the conservation of some endangered species through reintroduction programmes (Kleiman, 1996; WAZA, 2005), although they have not been without their difficulties (Beck, et al., 1991; Kleiman, 1996). Released zoo bred animals are slow to adapt to their natural environment, which is attributed to the safe and controlled environment the animals were reared in which does not sufficiently prepare them for the complexity of the wild (Kleiman, 1996). Real preparation for reintroduction should include exposing animals to shortages of food, parasites and disease, the threat of predation and adverse weather conditions, which to many are seen as unacceptable and even against animal welfare legislation (Beck, 1991; Veasey, et al., 1996b). Although species dependent, any reintroduction program requires an amount of pre-release conditioning, which for some species can be lengthy and expensive (Kleiman, Beck, Dietz, & Dietz, 1991). This has led to the argument that the high costs of reintroductions means that realistically, the limited resources available to the zoo community are best served by providing financial

support for in-situ conservation programmes (Hutchins & Conway, 1995; Kleiman, 1996). Reintroduction programmes are also rare and of the 418 endangered species mentioned in zoo Action Plans, only 19 have reintroduction plans (Barathay & Hardouin-Fugier, 2002). Providing a more complex, enriched environment, even without exposure to more naturalistic stressors can improve the abilities of animals to eventually adapt to their natural habitat (Robinson, 1998).

1.5.1 Evaluation of welfare in zoos

A scientific approach should be taken by zoological parks in the assessment and evaluation of their animals, and although the environment offers different challenges, the techniques successfully used in other captive settings should be adopted (Hill & Broom, 2009; Robinson, 1998). Although the evaluation of animal welfare is difficult it is generally recognised that a multi disciplinary approach is the best approach, including behavioural, physiological and psychological measures (Dawkins, 2004).

Behavioural studies have been highlighted as a useful method in assessing the welfare of animals in zoos, either through the presence of abnormal behaviours, stereotypies, or simply by comparison with the behaviour of conspecifics in the wild (Robinson, 1998). The use of cross-institutional behavioural studies has provided a means of increasing sample sizes to improve statistical reliability of zoo studies (Shepherdson, et al., 2004). The use of keeper surveys has also been an effective means of collecting data from large number of institutions (Hosey & Skyner, 2007; Inglett, French, Simmons, & Vires, 1989; Wielebnowski, 1999). Such studies have proved effective in revealing specific environmental effects on zoo animal behaviour, reproduction and welfare (Mellen, 1994).

One approach to welfare promotion requires animals in zoological parks be given the opportunity to behave as they would in the wild (Chamove & Anderson, 1989; Thorpe, 1967). This would require information on the behaviours of wild conspecifics, in order to take account of specific behaviours they may be highly motivated to perform. Time budgets of wild behaviour can also be a good guide as to which behaviours should be encouraged, although there may be difficulties in their interpretation (Veasey, et al., 1996a, 1996b). Knowledge about the life history of species is also vital because it informs the design of housing and social

environments. Animals have been categorised either as specialists that have evolved to exploit a specific niche or lifestyle, or generalists that are highly investigative and opportunistic and spend much of their time on the move (Morris, 1968). Although the dichotomy is an oversimplification, it can be a useful concept as it can be linked to an animal's natural ecology. A specialist has specific needs and if these are provided then the animal can cope well within captivity. For example, specialists include grazing or browsing herbivores that spend most of their time eating vegetation, or sloths (sub order Folivora) and koalas (*Phascolarctos cinereus*) with highly specialised diets (Robinson, 1998). Generalists however need to be given a stimulating environment that promotes their natural tendencies for exploration and curiosity, and includes the canines, mustelids and primates (Robinson, 1998). Generalists also tend to be omnivorous which suggest numerous and varied foraging techniques should be replicated in zoological parks.

The presence of stereotypies has been proposed as a useful indicator of poor welfare (Broom & Johnson, 1993) and increasing levels of stereotyping indicate a decreasing level of welfare (Dawkins, 1990). Reducing stereotypies has been identified as important in zoological parks and is one of the main reasons for their practice of environmental enrichment (Shepherdson, Mellen, & Hutchins, 1998; Young, 2003). However, the mechanisms that underlie stereotypies are complex and although their presence should be seen as a warning of potential welfare issues they should never be used as a sole indicator of poor welfare for an animal (G. Mason & Latham, 2004).

A combined approach using cross-institutional behavioural observations with non invasive physiological measures could provide an insight into the complex relationship between stress and environmental variables of a zoo environment (Shepherdson, et al., 2004). Any cross-institutional assessment studies should take account of various potentially confounding factors which may affect results (Mellen, 1994). These include variations in enclosure size and complexity (Carlstead, Fraser, Bennett, & Kleiman, 1999; Van Keulen-Kromhout, 1978); management routines (Bassett & Buchanan-Smith, 2007); and breeding and seasonal variation of behaviour patterns (Bassett & Buchanan-Smith, 2007; Carlstead & Seidensticker, 1991). Although the interpretation of cortisol studies are not without their difficulties with individual, age, sex and enclosure variation (Wielebnowski, 2003; Wielebnowski, et al., 2002), overall these studies indicate that non invasive monitoring of cortisol has

enormous potential for investigating how management and behavioural problems are related to animal wellbeing. If conducted under the right experimental conditions, this could allow researchers and managers to identify problem areas of zoo management and evaluate the efficacy of strategies designed to promote animal welfare and increased reproductive success.

Although a better understanding of the requirements of exotic species kept in zoological parks has led to an improvement in their welfare in recent years (Mench & Kreger, 1996), there is still relatively little known regarding their specific needs and requirements (Hill & Broom, 2009; Robinson, 1998). For the studies that have been carried out to date, there has been a strong bias towards mammals, and in particular primates (Melfi, 2005).

1.6 Primates in zoological parks

Primates are highly intelligent sentient beings that display a complex behavioural repertoire and require a stimulating environment (McCann, et al., 2007). This cognitive sophistication makes primates particularly susceptible to psychological stress arising from a variety of factors, not only through actual stressors but also in the anticipation of stress (Sapolsky, 2003, 2004). Primates also live in a variety of social systems, ranging from solitary, to pair living to multi-male / multi-female groups (Fuentes, 2007; Kappeler & van Schaik, 2002) and their social relationships within them are critically important (van Schaik & Aureli, 2000). When in captivity it is therefore essential that the appropriate conditions that satisfy their species-specific behavioural and social needs are met to ensure their wellbeing. For example, the separation of an individual that would naturally live solitarily is less of a welfare concern than the separation of an individual that is normally pair or group living, where a significant behavioural or physiological stress response is likely (Honess & Marin, 2006a).

Although the zoo environment has a number of environmental stimuli which have been identified as potential stressors, primates can be very adaptable and flexible (P. C. Lee, 1991) and living within a zoo environment should be within their range of adaptability (Poole, 1991). There can even be long term benefits of occasional and short-term stressors, particularly in early life, such as an increase in

vigilance and an increase in the number of natural behavioural patterns (Chamove & Moodie, 1990). In zoological parks many aspects of an animal's life history, such as feeding and reproduction, are beyond the control of the animals and are managed by humans (Hosey, 2008). Being confined can also reduce the ability of an individual to respond to aversive situations with appropriate escape or avoidance responses, which for social primates in particular, can be significant. These stressors can be in the form of proximity to predators, competing conspecifics, unfamiliar sounds, keeper interactions and perhaps one of the most prominent features, the presence of visitors (Carlstead & Shepherdson, 2000; Hosey, 2005).

There is evidence that some primates find life in zoological parks difficult with the performance of a variety of abnormal behaviours being reported and their causes needed to be identified and investigated (Hosey, 2005). It is essential that modern zoological parks can provide a suitable environment that a primate is able to adapt to. This is, however, not straightforward as there can be great variability in behaviour even within species to a range of captive environments. Comprehensive studies comparing the behaviour of different species in different captive and non-captive environments are needed in order to identify the animals' requirements. This includes how the different variables within a zoo environment, such as management, enclosure design and visitor presence interact with one another (Hosey, 2005).

1.6.1 Spider monkeys

Spider monkeys' social organisation is relatively unusual compared to most other mammals and primate species (Aureli & Schaffner, 2008), and this can potentially have an impact on their requirements for life in captivity. Although they live in multi-male/multi-female communities, a feature of many primate social groupings (Campbell et al., 2008), their communities are characterised by a high fission-fusion dynamic in which subgroup size varies due to the availability of and competition for resources (Kummer, 1971b). They spread out to forage in small subgroups or even alone in a flexible response that is dependent on the availability of food (Aureli & Schaffner, 2008; Aureli, et al., 2008). The constraints of captivity severely restrict opportunities for these fission-fusion events, but how this affects their social relationships within captive settings is not well understood and requires further investigation.

Living with high fission-fusion dynamics appears to reduce the need for the clear cut dominance hierarchies in females that are prominent in many primate species (Aureli & Schaffner, 2008). Female-female relationships in spider monkeys are generally low in value (Slater, Schaffner, & Aureli, 2009; van Schaik & Aureli, 2000), as females spend less time with other group members than males do and females disperse from their natal group on maturation (Di Fiore & Campbell, 2007). In contrast, male-male relationships are generally strong, and with the relative lack of predation pressure they form coalitions in order to protect their territories and protect their females and food resources from surrounding communities (Aureli & Schaffner, 2008), although these relationships can be variable and can have high risks for maturing males (Vick, 2008). These features of spider monkey social life provide important considerations for how they are managed in captive settings.

1.7 Thesis outline

I aimed to investigate social and external environmental factors influencing the welfare of captive Colombian black-faced spider monkeys (*Ateles geoffroyi rufiventris*). Data from two field sites in Colombia (Miller, Savage, & Giraldo, 2004) reveal that these animals face a real threat of extinction due to severe habitat destruction, and no current information exists to estimate numbers left in the wild (Savage, personal communication). Consequently, this species has been recently reclassified from vulnerable to critically endangered (Cuarón, Shedden, Rodríguez-Luna, & de Grammont, 2008). The successful management of the captive population is therefore crucial for potential future re-introduction programmes as well as for educational, research and captive breeding reasons (Kleiman, Allen, Thompson, & Lumpkin, 1996; WAZA, 2005). The following series of studies described in this thesis adopted, where possible, a multi-disciplinary approach with investigations involving physiological as well as behavioural measures that entailed measuring glucocorticoids in urine samples under a variety of social and external environmental conditions. The results of this study may have implications for management across different zoological parks for this critically endangered sub species (IUCN, 2008).

The first aim of my thesis was the validation of an enzyme immunoassay to quantify levels of cortisol excreted in the urine of spider monkeys. Previous studies

demonstrate that urine is an effective non invasive measure of hormones in Neotropical primates (French, et al., 1996; T. E. Smith & French, 1997a) and for reproductive steroid hormones in spider monkeys (Campbell, Shideler, Todd, & Lasley, 2001). The initial study involved the development and validation of a urinary cortisol assay for spider monkeys, which is presented in Chapter 2. The protocol involved biological validation which was determined by sampling urine across the diurnal phase of the 24-hour circadian cycle. Biochemical validation followed Reimers and Lamb's (1991) suggestions for appropriate immunoassay protocols and entailed demonstrating assay accuracy, assay specificity and precision. The validation allowed for the second aim of the thesis, which was to investigate the impact of a variety of stressors on urinary cortisol in the spider monkeys housed at Chester Zoo.

Recently, attention has been paid to assessing the impact of zoo visitors on the wellbeing of animals with mixed results, as some studies find no adverse effect of visitors on animals, whereas others report enriching or even negative effects (Chamove, Hosey, & Schaetzel, 1988; Davey, 2007; Hosey, 2000, 2005). Until the current research presented here, there had been no investigation of the physiological impact of visitors on a zoo primate species. The advent of foot-and-mouth disease provided a unique opportunity to assess the physiological impact of visitors on the spider monkeys. Chester Zoo was closed for a period of six weeks, during which time urine collection was ongoing thus allowing for the collection of physiological data during periods of no visitors, to contrast with periods of varying visitor levels. In Chapter 3 I present the physiological impact of visitors on the spider monkey HPA axis by assaying samples collected during various zoo visitor numbers, from zero when the zoological park was closed due to the foot and mouth outbreak, to very high when numbers exceeded 15,000 visitors in one day.

The next aim was to develop a questionnaire to determine whether any relationship between social structure and aggression could be determined across institutions. This was a research approach, which had been used previously, to assess patterns of aggression in golden-lion tamarins (Inglett, et al., 1989). This was subsequently sent out to 55 zoological parks and facilities around the world that housed four or more spider monkeys. A database was set up to record the number of aggressive acts, age and sex of the individuals involved, group structure at the time of the incident and finally the severity and context of that aggression. The

information from this questionnaire presented in Chapter 4 should identify when aggressive acts occur and with whom and whether this is linked with social management practices in captivity.

The final aim was to assess the impact of different social factors on HPA activity in the spider monkey group at Chester Zoo. Over the seven years of the study period there were a variety of social events that occurred and these were assessed in Chapter 5 by examining cortisol levels in the urine of the individuals of the group in the week prior to, during and the week following each event. This included the impact of aggressive behaviour, reproductive behaviour and the separation and reintroduction of members of the group over the study period.

A change in the social environment of an established group is potentially the most disruptive and traumatic event in captivity. In Chapter 6, a case study approach was used to investigate the significant social event of the replacement of the resident male at Chester Zoo with a new male from another zoological park. Physiological and behavioural data were collected from the whole group for a two week period prior to the arrival of the new male through to the successful integration of the new male six weeks later.

Thus the four data chapters in this thesis cover physiological responses to zoo visitors, physiological responses to several different categories of social events, a case study examining both behavioural and physiological responses to replacement of a breeding male, and the behavioural patterning of aggression in zoo-housed spider monkeys.

CHAPTER 2

METHODS – SUBJECTS AND ASSAY VALIDATION

2.1 Subjects and housing

2.1.1 Classification

The classification of *Ateles* has important implications regarding conservation priorities for both *in situ* and *ex situ* measures. Without a formal structure of names and an agreed system of usage, there can be no understanding of what exists to be conserved (Collar, 1997). New information regarding taxonomy from fields as diverse as physiology, genetics, behaviour and morphology are continually being published and to some degree the research is still in a state of flux (Collins, 2008; Nieves, Ascunce, Rahn, & Mudry, 2005).

The original classification of *Ateles* proposed four species (Kellogg & Goldman, 1944), including *A. geoffroyi* with nine sub species from Central America; *A. fusciceps* with two sub species in the Pacific coast region of north western South America; *A. belzebuth* with three separate sub species populations from Colombia through to the Amazon basin and *A. paniscus* with two distinct sub species from the Amazon basin. However, their classifications were based mainly on variations in pelage which differed considerably even within the proposed sub species. It has also been proposed that heterochromatism was responsible for the pelage variation and that all spider monkeys were in fact one polytypic species of *A. paniscus* (Hernandez-Camacho & Cooper, 1976; Hershkovitz, 1969, 1970). In a study based on morphological variation it was suggested that the genus instead should be separated into three distinct species of *A. paniscus, A. belzebuth* and *A. geoffroyi* (Froehlich, Supriantna, & Froehlich, 1991). However, Jacobs, Larson and Cheverud (1995) highlighted possible inaccuracies that arise from relying on pelage as a primary system for classification and demonstrated the importance of the genetic and developmental systems that underlie the phenotypic expression of pelage traits.

Using an analysis of the differences in karyotype in the populations of *Ateles* Medeiros, Barros and Pieczarka (1997) concluded that the genus should in fact be divided into four different species, but also indicated the necessity of a more

coherent taxonomic arrangement for *Ateles*. An analysis of phylogenetic relationships based on mitochondrial DNA (Collins & Dubach, 2000b) and then on nuclear DNA (Collins & Dubach, 2001) also concluded that there were four separate species of *Ateles* raising *A. hybridus* to species status. These were *A. paniscus, A. belzebuth*, *A. hybridus* and *A. geoffroyi,* including as subspecies the two former species *A. geoffroyi* and *A. fusciceps* from the Central American isthmus and the Choco region along the Pacific coast of Colombia and Ecuador, respectively (Kellogg & Goldman, 1944; Konstant, Mittermeier, & Nash, 1985). An assessment of the diversity of all Neotropical primates based on the diverse disciplines of taxonomy, biogeography, morphology and genetics (Rylands, et al., 2000) concluded that there were six separate species of *Ateles* making up a total of 16 taxa. These are *A. geoffroyi, A. chamek, A. paniscus, A. marginatus, A. belzebuth and A. hybridus.* However, the latest review of spider monkey taxonomy using morphological analyses (Froehlich, et al., 1991), molecular studies (Collins & Dubach, 2000b, 2001) and chromosomal analysis (Nieves, et al., 2005) all concur on the three species, *A. belzebuth*, *A. paniscus* and *A. geoffroyi*, with the latter two also supporting the species status of *A. hybridus.* While further research is required to clarify the taxonomic classification of spider monkeys, and particularly the status of the various sub species, it has been suggested that the latter four species taxonomy, supported by recent studies should be adopted to provide a consensus in all fields of spider monkey research (Collins, 2008). The American Zoological Society has adopted this taxonomy for use in its management of captive spider monkey populations and was also adopted for this study.

2.1.2 Distribution

Spider monkeys are found throughout Central and South America (Figure 2.1 and 2.2) with the northern, most distribution of *A. geoffroyi vellerosus* in Mexico ranging through to *A. chamek* in Bolivia and Peru in the south. Habitat preferences, behavioural characteristics, life history parameters and social structure are features of spider monkeys that were generally thought to be responsible for the present species distribution are reviewed in Collins and Dubach (2000a).

Figure 2.1 Distribution of *Ateles* across Central America based on Collins and Dubach (2000a).

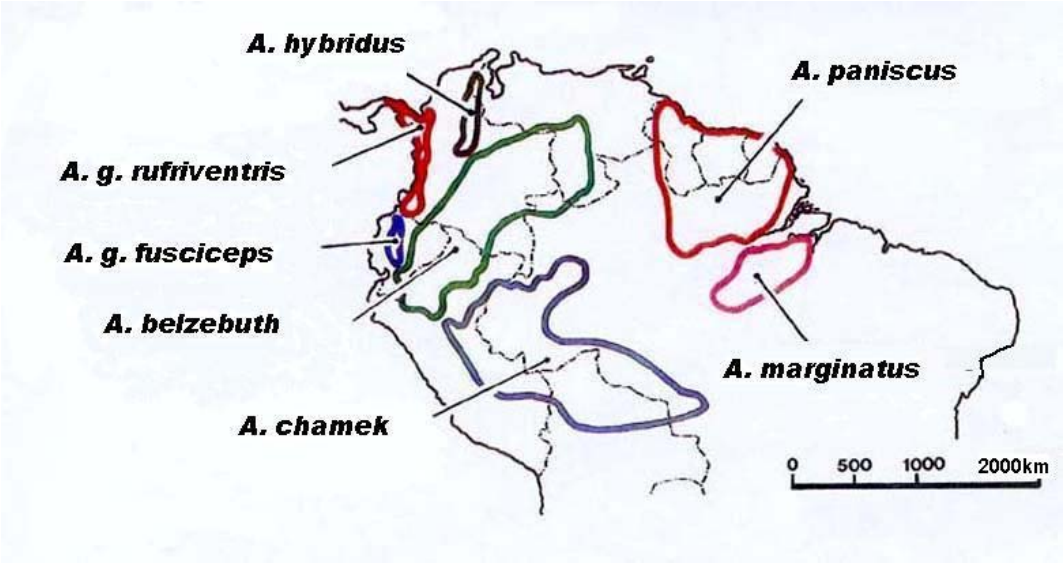

Figure 2.2 Distribution of *Ateles* across South America based on Collins and Dubach (2000a).

2.1.3 Conservation status

Spider monkeys are threatened by both habitat fragmentation and hunting pressure throughout much of their distribution (IUCN, 2006; Ramos-Fernandez & Wallace, 2008). They live primarily in the top canopy layers of low, humid, primary evergreen rainforest at elevations of less than 800m (Hernandez-Camacho & Cooper, 1976; Madden & Albuja, 1987). They are large-bodied and feed mainly on fruit necessitating large home ranges (Fedigan, Fedigan, Chapman, & Glander, 1988). In addition, spider monkeys have a long inter-birth interval of approximately three years and a slow development rate (Vick, 2008), which makes them particularly sensitive to hunting and deforestation.

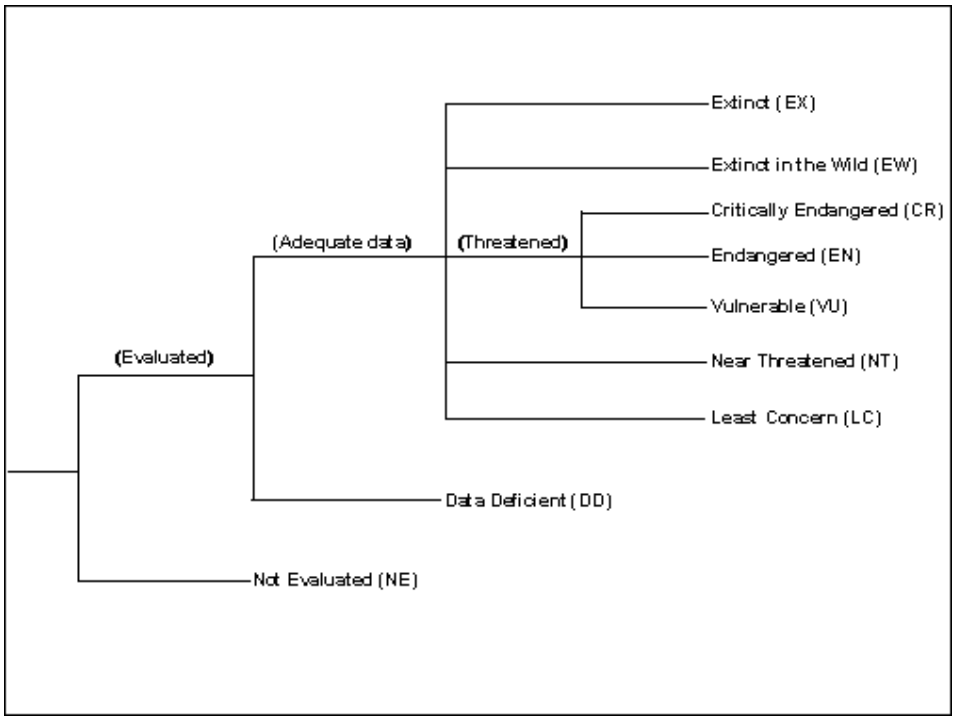

Figure 2.3 IUCN Categories of threatened species.

The IUCN identifies species/subspecies as critically endangered (CR) if they are facing an extremely high risk of extinction in the wild with a criteria of 50% risk of extinction within ten years or three generations; endangered (EN) if they face a very high risk of extinction in the wild with at least a 20% chance of extinction within 20 years or five generations; vulnerable (VU) if they face a high risk of extinction in the wild with the criteria of at least a 10% probability within 100 years; near threatened (NT) if close to qualifying for one of the above threatened categories

or likely to qualify in the near future, and finally as least concern (LC) when a taxon is not evaluated in any threatened category and are widespread and abundant (see Figure 2.3) (IUCN, 2006). Refer to IUCN (2006) for a full explanation of the criteria for conservation status.

2.1.4 Spider monkeys at Chester Zoo

The study group based at Chester Zoo was a breeding group of Colombian black faced spider monkeys. Formerly known as *Ateles fusciceps robustus* (Collins & Dubach, 2000a; Mittermeier, Rylands, & Coimbra-Filho, 1988*)* or as *A. fusciceps rufiventris* (Rylands, Groves, Mittermeier, Cortes-Ortiz, & Hines, 2005) following studies into variation of mitochondrial DNA they were recently reclassified as a subspecies of *A. geoffroyi*, and renamed as *A. geoffroyii robustus* (Collins & Dubach, 2000a, 2000b, 2001; Groves, 2001) or as *A. geoffroyii rufiventris* (Nieves, et al., 2005; Rylands, et al., 2005; Rylands, et al., 2000). Although the IUCN still classify them as *A. fusciceps spp. rufiventris* (Cuarón, Shedden, et al., 2008) in this study the sub species title of *A. g. rufiventris* has been adopted (see Table 2.1). Colombian black faced spider monkeys range from the western cordillera of the Andes from south western Colombia, northward on the west of the Rio Cauca to eastern Panama (Rylands, et al., 2005). Their conservation status has also recently been updated from vulnerable to critically endangered by the IUCN (Cuarón, Shedden, et al., 2008). For physical appearance see Figure 2.4.

The numbers of subjects varied over the study period from a minimum of eight to a maximum of eleven individuals (see Tables 2.2 and 2.3). Over the seven year study period the group changed as infants were born, sub adults were moved to other zoological parks or individuals died. Age classifications were categorised with adults over six years old (van Roosmalen & Klein, 1988), sub-adults from four to six years, juveniles from two to four years and infants under two years. This is s lightly different to some age classifications of spider monkeys in the wild (e.g. Shimooka, et al., 2008), as an individual was considered to be an adult if it had reached sexual maturity and zoo housed animals mature earlier than in the wild. During the study period two sub adult males were relocated to another zoological park in 2003, and the breeding male was relocated to another zoological park in 2008 with the

Table 2.1

Recognised species and sub species of Ateles (Collins, 2008) including their latest conservation status (IUCN, 2008).

Family	*Atelidae*			
Sub-family	*Atelinae*			
Genus	*Ateles*			
Species	[1]*A. belzebuth*	(White-bellied spider monkey)		Endangered
		Sub species	*A. belzebuth marginatus*	Endangered
			A. belzebuth chamek	Endangered
			A. belzebuth belzebuth	Endangered
Species	[2]*A. geoffroyi*	(Geoffroy's spider monkey)		Endangered
		Sub species	*A. geoffroyi geoffroyi*	Critically endangered
			A. geoffroyi azuerensis	Critically endangered
			A. geoffroyi frontatus	Vulnerable
			A. geoffroyi grisescens	Data deficient
			A. geoffroyi panamensis	Endangered
			A. geoffroyi ornatus	Endangered
			A. geoffroyi vellerosus	Critically endangered
			A. geoffroyi yucatanensis	Endangered
			A. geoffroyi fusciceps	Critically endangered
			A. geoffroyi rufiventris	Critically endangered
Species	[3]*A. hybridus*	(Variegated or brown spider monkey)		Critically endangered
		Sub species	*A. hybridus hybridus*	Critically endangered
			A. hybridus brunneus	Critically endangered
Species	[4]*A. paniscus*	(Guiana Spider Monkey)		Vulnerable

1 Boubli, et al., (2008; 2000a, 2001; 1991; 2005; 1993); Collins & Dubach (2000a, 2000b, 2001); Froehlich, et al., (1991); Nieves, et al., (2005); Sampaio, et al., (1993)

2 Froehlich, et al., (1991); Medeiros et al., (1997); Collins & Dubach (2000a, 2000b, 2001); Rylands et al., (2000); Nieves et al., (2005); Cuarón et al.,(2008)

3 Collins and Dubach (2000a, 2000b, 2001); Nieves, et al., (2005); Rylands et al. (2000; 2008); Urbani, et al., (2008)

4 Groves (1989); Froehlich, et al., (1991) ; Sampaio, et al., (1993); Medeiros et al., (1997); Collins and Dubach (2000a, 2000b, 2001); Nieves, et al., (2008; 2005); Mittermeier, et al., (2008)

immediate replacement with a new breeding adult male. In summary, the group was comprised of one adult male, who was changed in March 2008, four or five adult females, zero to two sub adult males, zero to two sub adult females and one to three infants (see Table 2.3).

Figure 2.4 Photo of *Ateles geoffroyi rufiventris* at Chester Zoo (Mar and All).

There were 16 births over the eight years, although half of these were either stillborn or did not survive the first 10 days. Of the remaining eight births, one individual was killed during a fight at six months in 2004 and one was euthanized due to poor health at 15 months, leaving six surviving offspring. Other changes in the group, which impact on the data presented in the forthcoming chapters, included a two year old that was born prior to the study period that had to be euthanized in 2000 after being lethally attacked by another spider monkey and in 2002 an adult female was euthanized following prolonged serious health problems.

Table 2.2

Individual spider monkeys that served as subjects throughout the study period.

ID	Date of birth	Sex	Age category during study	Dam	Sire	Change of group membership over study period
Ric	10.08.97	Male	Adult	unknown	Bru	Relocated – 07.03.08
Mar*	1970*	Female	Adult	unknown	Unknown	
Chr	30.09.88	Female	Adult	Mar*	Art	
Poy	03.09.89	Male	Adult	52AF4	52AF3	Introduced – 11.03.08
Mil	05.11.90	Female	Adult	Bla	Art	Death – 29.11.02
Zum	06.12.93	Female	Adult	Del	Fre	
Fay	22.02.94	Female	Sub adult/ adult	Mar*	Fre	
Joe	07.03.97	Male	Sub adult	Mar*	Fre	Relocated – 25.01.00
Ano	27.06.98	Male	Sub adult	Chri	Bru	Death – 25.07.00
Sul	30.10.99	Male	Sub adult	Mar	Ric	Relocated – 27.07.03
Bog	09.02.00	Male	Sub adult	Zum	Ric	Relocated – 27.07.03
Dor	24.03.00	Male	Infant	Fay	Ric	Death -16.04.01
MO2001	08.01.02	Male	Infant	Chr	Ric	DNS^{+} - 10.01.02
Naj	16.02.02	Female	Infant – adult	Fay	Ric	
CZ662	03.12.02	Female	Infant	Zum	Ric	DNS^{+} - 13.12.02
All	28.09.03	Female	Infant – sub adult	Chr	Ric	
CZ1409	15.04.04	Male	Infant	Zum	Ric	DNS^{+} – 15.04.04
CZ1888	13.10.04	Male	Infant	Fay	Ric	Death - 21.10.04
Pop	01.12.04	Male	Infant – sub adult	Mar*	Ric	
CO576	23.03.05	Male	Infant	Zum	Ric	DNS^{+} - 23.03.05
Bra	03.04.06	Male	Infant	Fay	Ric	Death - 15.10.06
CO6323	17.06.06	Female	Infant	Zum	Ric	DNS^{+} - 23.06.06
Syd	05.08.06	Male	Infant – sub adult	Chri	Ric	
CO7316	10.06.07	Male	Infant	Zum	Ric	DNS^{+} - 18.06.07
Win	17.08.07	Female	Infant	Mar*	Ric	
Fel	03.01.08	Male	Infant	Fay	Ric	

**wild caught*

$^{+}$*DNS = did not survive*

Table 2.3

Summary of group demographics over the eight year study period.

Year	Adult males	Adult females	Sub adult males	Sub adult females	Infants (surviving)	Max total
2000	1	5	2	0	3	11
2001	1	5	1	0	2	9
2002	1	5	2	0	1	9
2003	1	4	2	0	2	9
2004	1	4	0	1	2	8
2005	1	4	0	2	1	8
2006	1	4	1	2	2	10
2007	1	4	1	2	2	10
2008	1	5	2	1	2	11

2.1.5 Enclosure details

The animals were housed in an indoor enclosure measuring 138 m² (11.5m x 12m x to a height of 5m), which was furnished with many ropes, logs, branches, hammocks and various enrichment devices to encourage arboreal behaviour (Figures 2.5 and 2.6). The animals also had access to an outside enclosure measuring 950 m² (60m x 55m x 30m), which contained several trees, large shrubs and 8m pine poles interconnected with ropes or webbing (Figures 2.7 and 2.8). Plants growing in the enclosure included alder (*Alnus glutinosa*), laurel (*Prunus laurocerasus*), bamboo (*Sasa*), poplar (*P. canescens*), apple (*Malus domestica*), leylandii (*Cupressocyparis leylandii*) and buddleia (*Buddleja albiflora*). During the study period there were some changes to the outdoor enclosure with new poles and ropes installed.

2.16 Husbandry

The subjects had free access to both indoor and outdoor enclosures throughout the year, except during sustained cold periods of below 0°C when they did not have night-time access to the outside enclosure. The indoor area was connected to the outdoor area by two tunnels (3m x 0.8m x 0.8m), which passed over

Figure 2.5 Photograph of the indoor spider monkey enclosure at Chester Zoo.

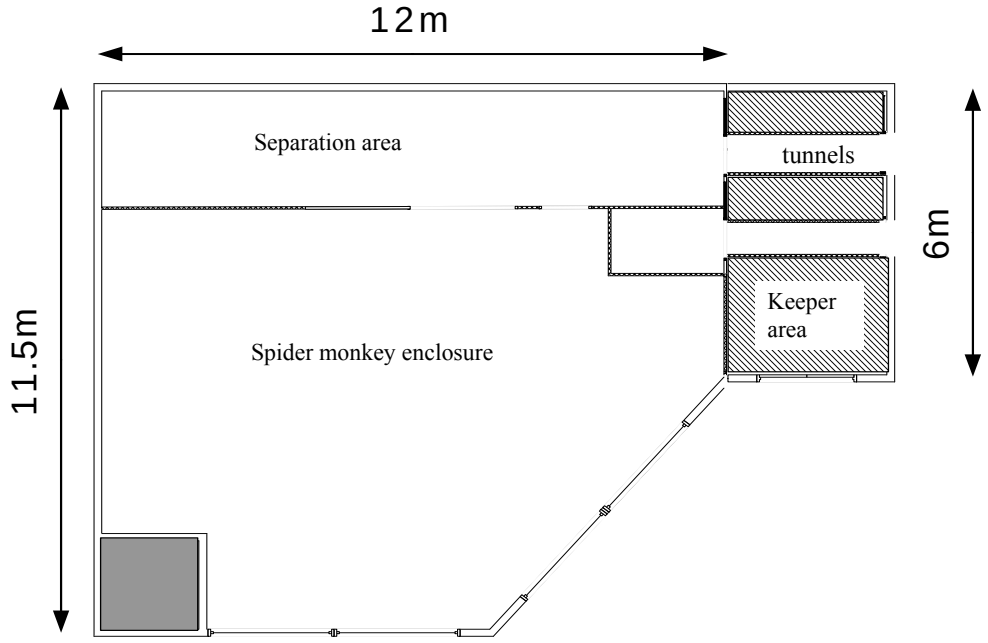

Figure 2.6 Plan of the general arrangement of the indoor enclosure at Chester Zoo showing the position of the tunnels used during urine collection and separation area.

Figure 2.7 Photograph of the spider monkey outdoor enclosure area at Chester Zoo.

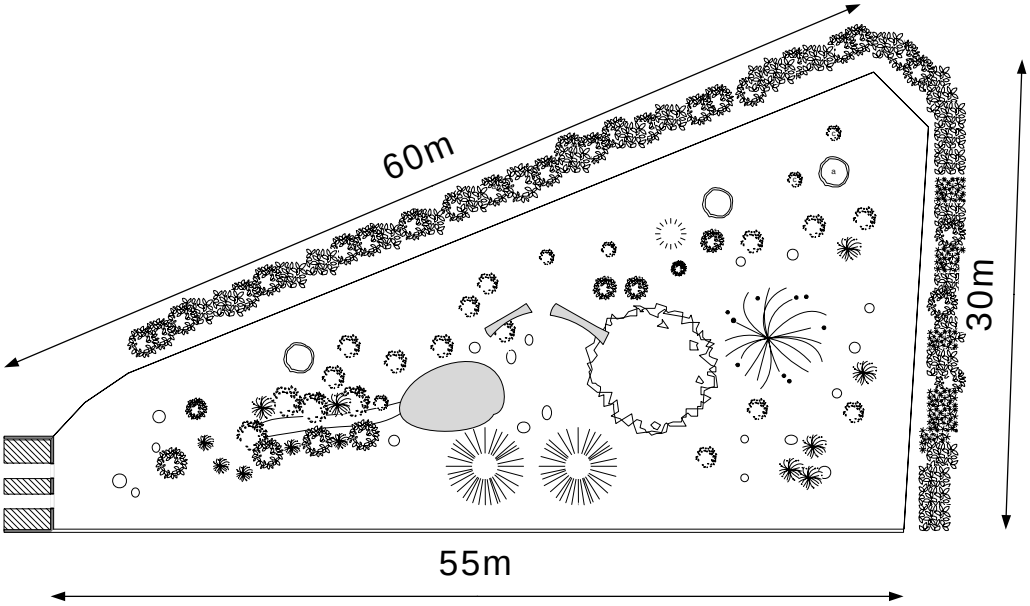

Figure 2.8 Plan of the general arrangement of the outdoor enclosure showing various vegetation, poles and pool and its relationship with the indoor enclosure.

a keeper area. The animals were normally maintained as one group; however there was the potential to separate animals at the back of the indoor enclosure when required (12m x 2.5m x 4m). This area was used in particular for separations or introductions of individuals and for particular husbandry reasons. Public viewing was achieved inside through four large viewing windows (2.4m x 2.2m each) and from along two sides of the outside enclosure. The tunnels and vegetation did provide a number of areas of privacy where the monkeys could escape from public view if they chose to do so.

The bedding consisted of a deep litter floor covering of wood bark and was cleaned daily, and entirely replaced every 12 – 18 months. Typically the monkeys received three feeds each day. The morning feed consisted of 600g of Primate pellet (Wildlife Feeds, England) and a supplemented protein such as eggs or mealworms. They were also fed approximately 2 kg of a variety of fruit and vegetables twice a day at varying times, initially supplemented with bread and Vionate vitamin and mineral powder (Sherleys) although this was later removed from their diet in February 2004. Food was presented in a variety of ways throughout the indoor and outdoor enclosure. For example, food could be placed in various hanging log feeders, baskets, sacks and on top of the roof to encourage natural arboreal feeding behaviour. Water was available *ad libitum* in the indoor and outdoor enclosures from pools, with the indoor pool changed daily.

2.2 General methods for urine collection

I collected urine three to four times a week between 0700 and 0800 hrs for all subjects for a period of seven years from February 2000 to September 2006 and then again from November 2007 to April 2008. A small proportion of samples were collected by other researchers over this time. Collection coincided with the daily husbandry regime when the monkeys were vacated from their inside enclosure for cleaning. This was advantageous in that no additional potentially stressful routine was required to collect the samples that could have affected the results. During this time the animals typically rested and waited in the tunnels over the keeper area (see Figure 2.6).

Table 2.4

Number of samples collected from each individual and for each corresponding study.

ID	Visitor impact	Social impacts	New male – case study	Total samples collected
	Chapter 3	Chapter 5	Chapter 6	
Ric	52	500	7	881
Mar*	23	416	32	724
Chr	42	486	33	875
Poy	n/a	n/a	13	13
Mil	15	129	n/a	189
Zum	25	295	8	503
Fay	22	314	24	598
Ano		n/a	n/a	32
Sul		n/a	n/a	259
Bog		n/a	n/a	297
Dor		n/a	n/a	51
Naj			11	293
All			21	118
Pop			7	56
Syd				15
Total	179	2140	156	4889

**wild caught*

I was able to stand under the tunnels and opportunistically collect urine in aluminium trays without requiring any formal training of monkeys, although successful voids were met with a vocal reward. This approach however did mean that urine samples were not collected from all individuals for each session. The samples were then transferred into labelled plastic vials, logged and immediately stored at –20˚C until assayed. The animals typically woke at around 06:45 when the lights came on; therefore the samples were nearly always the first void of the day. Samples were collected from all individuals with a total of 4889 samples collected over the whole study period for the various studies (see Table 2.4).

2.3 Assay validation

Previous studies have demonstrated that urine samples can be an effective non-invasive medium by which to measure hormones in Neotropical primates (French, et al., 1996; Schaffner & French, 2004; T. E. Smith & French, 1997a) and specifically in spider monkeys (Campbell, et al., 2001) [typically using an enzyme-immunoassay (EIA)]. There is no previous literature on using EIA for quantifying cortisol in spider monkeys, therefore it was necessary to immunologically and biologically validate the assay (Buchanan & Goldsmith, 2004). The immunological validation is assessed through the demonstration of specificity, accuracy, precision and sensitivity (Diamandus & Christopoulos, 1996; Reimers & Lamb, 1991).

Specificity is the assay's freedom from interference from other substances other than the one being studied. It can be determined by examining the parallelism of serial dilutions of the study samples with the standard solutions to establish whether the substance in the samples is immunologically identical to the substance in the standard solution (Reimers & Lamb, 1991).

Accuracy of an assay can be determined by adding known amounts of the target hormone to several samples. If the assay is quantitatively accurate the quantity of the hormone that the assay recovers (i.e. measures) should equal the amount added. The percentage of hormone that is recovered can then be calculated. This can be demonstrated by plotting the quantity added against quantity recovered, and the slope of the line should approximate 1 (Reimers & Lamb, 1991). Precision is a measure of the assay's variability and is expressed as the coefficient of variation (CV) based on replicate measurements of a known sample (quality control) (Reimers & Lamb, 1991). Both the within assay variation (intra-assay) and between assay variation (inter-assay) should be reported. Sensitivity is the smallest amount of unlabelled hormone that can be distinguishable from the absence of hormone (Reimers & Lamb, 1991). To improve sensitivity it may be necessary to increase the volume of the sample, incubation time or temperature.

The biological validation of an assay can be demonstrated by determining whether the assay detects biologically meaningful changes in hormones. For example, it can involve verifying the presence of diurnal variation in the excretion of cortisol metabolites. Plasma cortisol is known to follow a diurnal pattern of excretion

in diurnal mammals whereby cortisol levels peak in the early morning when the animals awaken and then gradually decrease throughout the day to reach the lowest point when the animals retire (Coe & Levine, 1995; Crockett, et al., 2000) and has been demonstrated in the plasma of many primate species (Abbott, et al., 2003; M. R. Clarke, Harrison, & Didier, 1996; Gust, et al., 2000; Saltzman, Schultz-Darken, & Abbott, 1996). A similar diurnal pattern of urinary cortisol excretion has also been reported in several species (Anestis & Bribiescas, 2004; Coe & Levine, 1995; McCallister, Smith, & Elwood, 2004; Muller & Lipson, 2003; T. E. Smith & French, 1997a).

2.3.1 Aims

The first aim of this study was to validate an EIA for the measurement of cortisol in the urine of Colombian black-faced spider monkeys (*Ateles geoffroyi rufiventris*) in order that the activity of their HPA axis could be measured. This is the first time an enzyme-immunoassay has been carried out for the genus of Ateles. Immunological validation is assessed through the demonstration of accuracy, specificity, precision and sensitivity (Diamandus & Christopoulos, 1996). To ensure the assay detects biologically meaningful changes in cortisol the validation also evaluated whether levels of cortisol excreted in the urine followed the typical circadian pattern of diurnal animals evident in the plasma and urine of many other primate species (Abbott, et al., 2003; M. R. Clarke, et al., 1996; Gust, et al., 2000; Saltzman, Schultz-Darken, Wegner, Wittwer, & Abbott, 1998).

2.3.2 Methods

Urine samples were collected from all members of the spider monkey group throughout the study period. Change in group composition are summarised in Table 2.2 with samples assayed from a total of eight adults. The demographics of the group are also summarised in Table 2.3.

Levels of excreted cortisol were measured in the selected urine samples using a modified EIA applied previously by Smith and French (1997a) in Wied's marmosets (*Callithrix kuhlii*) to quantify excreted urinary cortisol. The cortisol antibodies and horseradish peroxide conjugated cortisol were supplied by the University of California (Davis, USA) while all other chemicals were supplied by

Sigma-Aldrich (Poole, Dorset, UK). The assays were carried out on 96 well Microtiter plates (Maxisorp, NUNC™) and samples were run in duplicate (See Appendix A for template). The stock solutions for the cortisol antibody [R4866, raised against a steroid bovine albumin (BSA) in rabbit] and the cortisol horseradish peroxide (Hrp) were stored in dilutions of 1:50 in deionised water and 1:100 in EIA phosphate buffer solution (PBS) respectively, and stored in the freezer at -20°C in small aliquots. The antibody was diluted to 1:12,000 in 0.05M coating buffer for the purposes of the assay (1.59 g Na_2CO_3, 2.93g $NaHCO_3$, 1L dH_20, pH 9.6). This antibody has reported to have cross reactivities of 96% with prenisolone, 66% with prednisone, 60% with cortisone, 2.5% with corticosterone and < 1% with various other steroids (Ziegler, et al., 1995).

The antibody (Ab) was made up to the working dilution of 1:12,000 and 50 µl was coated to each of 94 wells. The two remaining wells were used to determine non-specific binding (NSB) and were coated with 50 µl of carbonate buffer to act as a control for non-antibody binding. The plates were then tapped eight times on each side to allow an even coating of the antibody. They were then covered with an adhesive plate sealer and incubated for 12-18 hours in the refrigerator at around 4°C. The following day all plates, samples and buffers were allowed to reach room temperature. Eppendorf tubes (1.8 ml volume) were then labelled and arranged in the tube racks corresponding to the template (see Appendix A) of the samples to be run. The samples were run at a working dilution of 1:512. This dilution was made by diluting samples to 1:64 by mixing 10 µl of the urine sample newly defrosted at room temperature in 630 µl of distilled water, followed by making a 1:8 dilution by adding 100 µl of the 1:64 dilutions to 700 µl of distilled water. Distilled water was maintained at room temperature. The working dilution had been previously calculated as that dilution which showed approximately 50% binding against the standard curve, following previous protocols (McCallister, et al., 2004; T. E. Smith & French, 1997a).

The excess antibody was then emptied before each plate was washed six times using the plate washer, three times in each direction to minimise potential drift across the plate (10:1 EIA wash solution: 87.7 g NaCl [1.5M], 5 ml Tween 20 [0.5%], 1L dH_20, 350 µl/well). Immediately after the plates were washed, 50 µl of EIA PBS were added to all wells, followed by the 50 µl of the standards diluted in

dH_20 (n = 10; 1.95-1,000 pg; Sigma, St. Louis, MO) and the samples (50 µl) in duplicate in the appropriate wells. Finally, 50 µl of cortisol Hrp (Batch#12/18/03) diluted to the working solution of 1:22,000 were added to all wells. The plates were then sealed again and left to incubate for 2.5 to 3 hours at room temperature in the dark. The assays were carried out in a laboratory and the temperatures were maintained at a steady 21°C.

Following incubation the plates were washed six times as before, and then 100 µl of the EIA substrate solution were added to all wells (12.5 ml EIA citrate buffer [0.05 m, pH 4.0:9.61 g citric acid (anhydrous), 1 l dH_20], 125 µl EIA ABTS [40 Mm, 2,2'AZINO-bis (3 ethylbenzthmoline-6-sulfonic acid) Diammonium salt], 0.329 g ABTS, 15 ml dH_20, pH to 6.0; 40 µl EIA H_20_2 [2.0%, 0.5 M, 500 µl H_20_2 (30%) 8 M, 7.5 ml dH_20]). The plates were then left on a plate shaker to develop before being read using a microplate reader (Dynatech MR700) and the software Revelation version 4.22. The plates were left until the optimal density at 405 nm for the control wells with no hormone (Bo) measured 1.0. Any sample with a coefficient of variation (CV) greater than 10% was repeated.

A stock urine pool was made up for the purpose of providing quality control samples to run on each plate. The quality controls were used to calculate the intra- and inter-assay CV (see below). Two different pools were made up altogether, one for the assay validation and visitor effect study (Pool A, see Table 2.5 and Chapter 3 respectively) and one for the social impact study and case study (Pool B, see Table 2.4 and Chapters 4 and 5).

Pool A was made up by taking 100 µl from each of 35 different samples taken from each of the group members that took part in the immunological validation and visitor study (see Table 2.5). An individual contributed either five or ten samples to the pool depending on their contribution to the overall study. Overall, pool A was made up of 10 samples from the adult male and 25 from the adult females.

Pool B was made up by taking 100 µl from each of 88 different samples taken from each of the group members that were present during the social impact study. Fourteen samples were selected from each adult that remained in the group throughout the study period, six samples from an adult that was only present during part of the study and six from each of the two sub adults. In summary, the pool was made up from 62 samples from the adult females, 14 from the adult male and 12 from the sub adult males.

Table 2.5

Information on the pools used in the cortisol EIA for the different studies.

Pool	Number of samples	Time period	Number and sex of animals	Experiment
Pool A	35	Feb 2000 – May 2003	5 (1 M and 3 F)	Validation
Pool A	35	Feb 2000 – May 2003	5 (1 M and 3 F)	Visitor effect Chapter 3
Pool B	88	Feb 2000 – Dec 2003	8 (3 M and 5 F)	Social impact / Case study Chapter 5/6

To compute inter- and intra-assay variation, a high quality control of 1:128 dilution and a low quality control of 1:1024 dilution for the appropriate Pools A and B was used for each plate. These dilutions were chosen since they represented approximately 33% and 66% binding of the AB. These dilutions were made up when required and stored in the freezer at -20°C.

2.3.2.1 Immunological validation method

An enzyme-immunoassay is based on an immunological reaction in which the analyte of interest binds to a specific antibody. This binding process can be affected by variables capable of causing imprecision and inaccuracy, such as interfering substances that could cross-react with the samples. Immunological validation is necessary and followed Diamandis and Christopoulos (1996) suggestions for appropriate immunoassay protocols demonstrating accuracy, specificity, precision and sensitivity.

Assay accuracy was determined by adding a low (1:2048), a medium (1:1024) and a high concentration (1:32) of Pool A to six serial diluted commercial standard samples [n = 6, 500, 250, 125, 62.5, 31.25, 15.6 pg/50 ul)] to ascertain sample recovery. To determine assay specificity, two displacement curves of halving dilutions of a urine pool A (see Table 2.5), ranging from 1:32 to 1:4086, were compared with a displacement curve of cortisol standard preparation to determine parallelism. The F-statistic was used to compare the slopes of the linear regressions of the displacement curves.

Precision was ascertained for each urine Pool separately, by monitoring inter- and intra assay coefficient of variations by using two quality control samples [a high quality control at around 65% binding (1:1024), and a low quality control at around 37% binding (1:128)] on all plates assayed for each part of the study. The intra-assay variation was calculated by averaging reported CV for high and low quality controls on each plate. The inter-assay CV was determined by dividing the overall standard deviation of the quality control CVs by the overall mean multiplied by 100 to get a percentage. Target values for intra- and inter assay CVs are 5 and 10% respectively, although values up to 10 and 20% respectively are acceptable (Diamandus & Christopoulos, 1996). Sensitivity was calculated as a mean from all assays of the lowest concentration of cortisol on the 90% binding point of the standard curve.

2.3.2.2 Immunological validation results

For accuracy the recovery of the commercial standard preparations added to the low concentration pool was 123.6% ($r = 1.0$; $Y = 1.26X + 0.99$; $p < 0.0001$), the medium concentration pool was 104.8% ($r = 1.0$; $Y = 1.08X - 3.91$; $p < 0.0001$) and the high concentration pool was 97.8% ($r = 0.993$; $Y = 0.97X - 4.51$; $p < 0.0001$).

For specificity two separated serial dilutions of the urine pool A gave displacement curves that were parallel to the serial dilutions of the commercially prepared cortisol standards [F (2, 16) = 0.53, NS; F (2, 20) = 2.94, NS]. Results are calculated as the ratio of the antibody-analyte complexes of standards or samples that have bound (B) versus that bound at zero concentration or maximum binding (Bo) and is expressed as % B/Bo (Figure 2.9).

For precision the intra-assay variation for the validation and visitor effect study (Chapter 3, Pool A) was 5.67% (n = 8) and 4.40% (n = 8) for the high and low quality control pools respectively. For the social impact study (Chapter 5, Pool B) the assays were carried out over three batches with new standards and controls used each time. For batch one intra- assay variation was 6.03% (n = 33) and 6.16% (n = 33), for batch two 8.96% (n = 39) and 5.93% (n = 39) and for batch three 7.09% (n = 45) and 3.24% (n = 45) for the high and low quality control pools respectively. Finally, for the new male case study (Chapter 6, Pool B) intra-assay variation was 5.59% (n = 5) and 3.83% (n = 5) for the high and low quality control pools respectively.

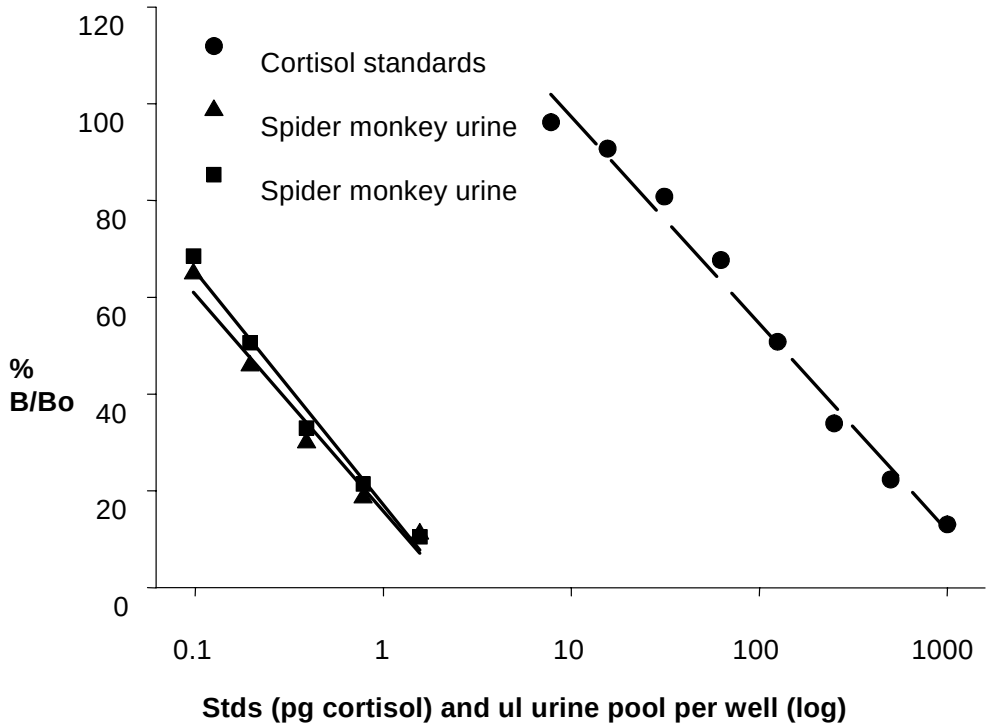

Figure 2.9 The binding ratio (%B/Bo) of two serial dilutions of the spider monkey urine Pool A and the cortisol standards to demonstrate parallelism.

The inter-assay variation for the validation and visitor effect study (Chapter 3, Pool A) was 2.03% (n = 8) and 11.51% (n = 8) for the high and low quality control pools respectively. For the social impact study (Chapter 5, Pool B) the assays were carried out over three batches with new standards and controls used each time. For batch one the inter-assay CVs were 15.67% (n = 33) for the high and 19.81% (n = 33) for the low quality control pools, for batch two 19.58% (n = 39) for the high and 18.21% (n = 39) for the low, and for batch three 17.7% (n = 45) for the high and 20.9% (n = 45) for the low quality control pools. Finally, for the new male case study (Chapter 6, Pool B), the inter-assay CV was 15.5% (n = 10) and 15.6% (n = 10) for the high and low quality control pools respectively. The assay sensitivity was 3.95 pg.

2.3.2.3 Biological validation method

Five adult spider monkeys from the Chester Zoo group contributed a total of 53 urine samples to the circadian assessment. Samples were collected opportunistically from 08:00 hrs to 18:00 hrs for three days over a seven-day period from the five adult

subjects (Table 2.6). The diurnal period was divided into five time blocks of two hours and the samples were collapsed for each subject across the three collection days. The mean concentration of urinary cortisol was calculated for each animal in each time slot and analysed using a one-factor repeated measure of analysis of variance. This test was followed by a linear trend analysis to determine if there was a significant decreasing pattern over time of day (Keppel, 1993).

Table 2.6

Number of samples used for each time block for the circadian rhythm validation.

Time	Total number of samples	Mean number of samples per subject
06:00 – 07:59	12	1.7
08:00 – 09:59	13	1.9
10:00 – 11:59	11	1.6
12:00 – 13:59	12	1.7
14:00 – 15:59	12	1.7

2.3.2.4 Biological validation results

A circadian variation in urinary cortisol excretion was demonstrated when samples collected over three days and across an 8-h period were analysed (Figure 2.10). The assay was thus effective in detecting diurnal variation in cortisol levels [$F(4, 16) = 4.59$, $p < 0.001$] confirming that cortisol excreted in the urine, as measured in my assay accurately reflects levels of cortisol circulating in the plasma. Trend analyses revealed a significant decreasing linear trend across the five time periods [$F(1, 4) = 9.75$, $p < 0.035$].

2.3.2.5 Creatinine assay

The hormone concentration for each sample was corrected for creatinine concentration using a modified Jaffe end-point assay (Burtis & Ashwood, 2001). To identify the most appropriate dilution an initial assay was run using a series of urine

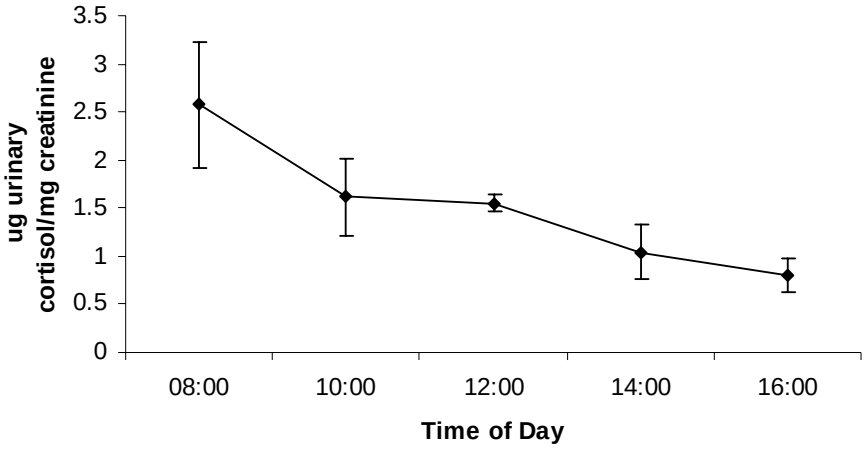

Figure 2.10 Mean ± SEM levels of urinary cortisol across the time of day.

dilutions from a high dilution of 1:2 to a very low dilution of 1:2048. Based on the latter assays, the working dilution for creatinine assays was run at a dilution of 1 part urine in 200 parts distilled water.

The working dilution was made in two steps involving a 1:64 dilution followed by a 1:3.125 dilution. The urine samples stored at -20°C were defrosted at room temperature prior to assaying and thoroughly mixed for around 10 seconds using a vortex mixer. To create the 1:64 dilution, 630 µl of distilled water was pippetted into individually labelled 1.5ml eppendorf tubes, added to 10µl of the sample and mixed thoroughly. For the second step 235 µl of the 1:64 dilution was mixed with a further 500 µl of distilled water in another labelled 1.5ml eppendorf tube to form the working dilution. For each plate a maximum 28 samples could be run in duplicate (See Appendix B for template).

The inter plate reliability was assessed by running on each plate a high quality control (HQC) of 1:128 dilution and a low quality control (LQC) of 1:1024 dilution of the appropriate pools. Again the dilutions were measured out in two steps, with a one to one dilution of the 1:64 urine dilution with distilled water for the HQC and a one to sixteen dilution of the 1:64 urine dilution with distilled water for the LQC.

Creatinine assays were carried out using 96 well non bonding microtiter plates (Maxisorp, NUNC™). Standards (n = 4, 6, 3, 1.5, 0.75 µg / 200 µl, Sigma)

and samples (at 1:200) were diluted in dH_20 and 200 µl pipetted into the appropriate wells in duplicate. Control wells contained 200 µl of dH_20 as an indicator of nonspecific binding. For each plate a mixture of 5ml of NaOH (0.75M) and 5 ml of picric acid was required, with 100 µl of the mixture added using a multi pipette to all wells except the control wells. The plate was then placed on the shaker for 1-2 minutes then read using the software Revelation version 4.22 on a microplate reader (Dynatech MR700), when the optical density at 490 nm of the top standard measured around 1.7. All urinary cortisol concentrations were expressed relative to creatinine (ug urinary cortisol / mg Cr).

2.3.3 Summary

An enzyme-immunoassay was successfully developed and validated to quantify urinary cortisol in spider monkeys. Immunological validation of the assay was achieved by showing specificity, accuracy, precision and sensitivity. The biological validation was confirmed with the detection of a typical diurnal pattern of cortisol excretion in the urine, which is evident in the plasma of primates (Coe & Levine, 1995; Czekala, Lance, & Sutherland-Smith, 1994; T. E. Smith & French, 1997a).

By developing a biologically valid assay to quantify cortisol I have added to the growing number of research studies that use physiological indices as a tool for measuring potential stressors and biological events (Boinski, et al., 1999; Crockett, et al., 2000; Dettling, et al., 2002; Whitten, et al., 1998; Ziegler, et al., 1995). Recently, studies using faecal steroid assays have assessed the relationship between puberty and dispersal in wild female muriqui monkeys (*Bracyteles arachnoids*) (Strier & Ziegler, 2000) and ovarian cycles in Geoffroy's spider monkeys (*A. geoffroyi*) (Campbell, et al., 2001). My findings contribute to these advances in the study of steroid hormones in Ateline primates. Collectively, this research is relevant for the captive breeding and management of New World monkeys as it provides a mechanism to gain valuable information regarding general welfare and reproductive competence, as well as encouraging researchers to explore more refined questions, such as the impact of the zoo environment on physiology.

To conclude, I have validated an enzyme-immunoassay to quantify levels of urinary cortisol in spider monkeys. The biochemical technique can be applied to

assess the relationship between various stressors and a physiological index in a primate species.

CHAPTER 3

EVIDENCE THAT ZOO VISITORS INFLUENCE HYPOTHALAMIC PITUITARY ADRENAL AXIS ACTIVITY IN SPIDER MONKEYS (*ATELES GEOFFROYI RUFIVENTRIS*)

3.1 Introduction

3.1.1 Factors effecting welfare of zoo-housed animals

Animals in captivity are exposed to a variety of potentially harmful stressors. These include environmental sources of stress related to housing conditions such as artificial lighting, aversive sounds, odours, substrate and extremes of temperatures, as well as confinement-specific stressors such as reduced retreat space, forced proximity to humans, restricted movement, abnormal social groupings, reduced feeding opportunities and other restrictions on opportunities for natural behaviour (Morgan & Tromborg, 2007). Animals in captivity also have a reduced amount of control over their environment and an increased amount of predictability (Carlstead, 1996). It is this lack of control and variations in predictability that are potentially the greatest stressors for animals in captivity (Bassett & Buchanan-Smith, 2007; Sambrook & Buchanan-Smith, 1997; Wiepkema & Koolhaas, 1993).

A zoo environment has been identified as being unique compared to other captive environments and is characterised by the combination of three specific factors (Hosey, 2005). Firstly, the physical available space for animals in zoological parks is much smaller than they would normally range over in the wild. The impact of this restricted space on the welfare of the animals is however complex. While sufficient quantity and quality of space must be provided to enable appropriate species-specific behaviours the provision of resources makes comparisons with the wild difficult. The second is that most aspects of a zoo animal's life are managed to some degree by humans. Their accommodation, feeding, group composition, health and reproduction are all to a greater degree out of their control. Finally the constant presence of a large number of unfamiliar humans is also unique to a zoo environment. The impact of visitors on zoo animals has been investigated with a

review of studies showing how various visitor characteristics can have an effect (Davey, 2007).

3.1.2 Zoo visitor studies

There has been conflicting evidence regarding the effect that visitors have on the lives of captive animals (Hosey, 2000; Hosey, Melfi, & Pankhurst, 2009b). For example, while no significant effect was found on the behaviour of six species of felids (Margulis, Hoyos, & Anderson, 2003), or captive cheetahs (*Acinonyx jubatus*) (O'Donovan, Hindle, McKeown, & O'Donovan, 1993), visitors did have an effect on captive Indian leopards (*Panthera pardus*) (Mallapur & Chellam, 2002) and jaguars (*Panthera onca*) (Sellinger & Ha, 2005). However, the vast majority of visitor studies have been carried out on primates with studies also showing a variety of effects (Davey, 2007). Some researchers have reported no effect of zoo visitors on primates (Adams & Babladelis, 1977; Synder, 1975), whereas other researchers report an enriching effect. Cook and Hosey (1995) reported how chimpanzees (*Pan troglodytes*) voluntarily interacted with visitors in order to try and obtain food, while Fa (1989) reported positive effects when visitors threw food towards green monkeys (*Cercopithecus aethiops*), although such behaviour is likely to have negative consequences towards their general health. The majority of studies however appear to demonstrate negative effects to various degrees (Chamove, et al., 1988; Glatston, Geilvoet-Soeteman, Hora-Pecek, & Van Hooff, 1984; Hediger, 1969; Mallapur, Sinha, & Waran, 2005; Mitchell, Obradovich, Herring, Dowd, & Tromborg, 1991; Skyner, Amory, & Hosey, 2004; Wells, 2005) ranging from an increase in locomotion in a variety of species (Hosey & Druck, 1987), an increase in aggression in mangabeys (*Cercocebus galeritus chrysogaster*) (Mitchell, Herring, et al., 1991), increasing aggression and stereotypic behaviour in gorillas (*Gorilla gorilla*) (Wells, 2005), pied tamarins (*Saguinus bicolor bicolor*) (Wormell, Brayshaw, Price, & Herron, 1996), and mandrills (*Mandrillus sphinx*) (Chamove, et al., 1988), increases in abnormal behaviours in lion tailed macaques (*Macaca silenus*) (Mallapur, et al., 2005) and self-harming behaviour in gibbons (*Hylobates pileatus*) (Skyner, et al., 2004). A prevalence of aversive consequences may be linked to a closer taxonomic relationship between visitors and other primates and possibly linked with more familiarity in communicative signals (Hosey, Melfi, & Pankhurst, 2009a).

3.1.3 Factors influencing the impact of visitors on zoo primates

There are many factors that could be influencing the impact of visitors on zoo animals' wellbeing which have been outlined by Hosey (2000). Firstly, there appears to be a considerable amount of inter-species variation (Chamove, et al., 1988; A. S. Clarke & Mason, 1988; Mitchell, Herring, et al., 1992; Wormell, et al., 1996) that may be explained by the degree to which an animal may see the human as a threat. This perception can be related to the animals body size, its social organisation, species typical responses to environmental events and the extent of habituation to humans (Hosey, 2005). Secondly, the design of the enclosure can also be significant (Carlstead & Shepherdson, 2000; Chamove, et al., 1988; Glatston, et al., 1984; Hosey, 2000; Mitchell, et al., 1990; Wormell, et al., 1996). The size and complexity of the enclosure space will affect how the animal will respond to visitors, with larger more complex exhibits allowing more retreat space and opportunities for individuals to remove themselves from the view of the public (Hosey, 2005). Thirdly, the way in which visitors view the animals can also be an important factor with the size of viewing windows (Blaney & Wells, 2004), height of viewing (Chamove, et al., 1988) and position of exhibits (Margulis, et al., 2003; Mitchell, et al., 1990) all potentially having an impact. Finally, the behaviour of visitors can also make a difference, with noise, size of crowd and activity level all impacting on the organisms (Birke, 2002; Hosey, 2000; Hosey & Druck, 1987; Mitchell, Obradovich, et al., 1991).

3.1.4 Using HPA activity to assess visitor impact

Although there is a general consensus that visitors can have a negative impact on zoo animals, there is sufficient inconsistency to warrant further study (Hosey, 2000). Previous studies exclusively relied on behavioural indices to assess the impact of visitors on animals, in particular monitoring changes in affiliative and abnormal behaviours (Davey, 2007). While this can be an effective method, it can be difficult to interpret how behavioural changes can affects an animals' welfare, particularly regarding the presence of abnormal behaviours (G. Mason & Latham, 2004) (see Chapter 1, section 1.3.4). The use of physiological measures as a means of assessing the stress response provides additional evidence and insight into the effect of visitor numbers on individuals. To my knowledge, only one previous study has used physiological indices to assess visitor impact (Kalthoff, Schmidt, & Sachser, 2001).

This used salivary cortisol and behaviour in several mammal species including rhinos, although no significant relationship was found between GC levels and visitor numbers. My study attempted to increase the understanding of visitor effect by assessing the relationship between visitor numbers and one aspect of an animal's physiology; activity in the HPA axis.

Previous studies have demonstrated that the analysis of urine and faeces can provide an effective method of measuring reproductive steroid metabolites in Neotropical primates, such as marmosets and tamarins (French, et al., 1996; T. E. Smith, Schaffner, & French, 1997; Ziegler & Snowdon, 2000; Ziegler, Wegner, Carlson, Lazaro-Perea, & Snowdon, 2000). Campbell et al. (2001) have used urine and faecal analysis to investigate levels of pregnane-diol 3α glucuronide and estrone conjugates in the ovarian cycles in female spider monkeys (*Ateles geoffroyi*).

Cortisol, another steroid hormone, is the end product of HPA activity and is an effective marker for assessing physiological stress in captive animals (Boinski, et al., 1999; Crockett, et al., 2000; T. E. Smith & French, 1997a; Whitten, et al., 1998; Ziegler, et al., 1995; see Chapter 1, 1.4.10). Therefore measurement of cortisol in the urine can potentially provide information about the physiological response of a non-human primate to a potential stressor, such as visitors.

3.2 Aim

The aim of my study was to investigate the physiological impact of visitors on the spider monkey HPA axis by quantifying levels of urinary cortisol in samples collected during days of varying visitor numbers (i.e. 0 to 16,500 visitors). The relationship between concentrations of urinary cortisol and actual visitor numbers was then investigated. I predicted that if visitors adversely impacted the animals then a positive relationship would be identified between urinary cortisol and visitor numbers.

In February 2001 foot and mouth disease (FMD) appeared in the UK with a devastating impact on all livestock industries (Mepham, 2004). Consequently, strict restrictions were imposed on the movement and handling of animals throughout the country. Farms, zoos and safari parks were closed to all but essential staff for the duration of the outbreak. Chester Zoo was closed for a total of six weeks from

February 25th, 2001 to April 6th, 2001. During this time only essential keeping staff members were allowed into the zoological park. The closure of Chester Zoo provided a unique opportunity to collect data for a period when there were no zoo visitors.

3.3 Method

3.3.1 Subject and housing

The study involved five adult females, one adult male and three juvenile males from a breeding group of Colombian spider monkeys (*Ateles geoffroyii rufiventris*) housed at Chester Zoo (Table 3.1). The animals had access to both the indoor enclosure throughout the study, apart from the adult male who due to management reasons was separated on his own in the separation pen inside (for details of the enclosure and husbandry see Chapter 2, section 2.16 and Figures 2.4-2.7).

3.3.2 Procedure

3.3.2.1 Urine collection

Urine collection was conducted three to four times a week between 0700 and 0800 hrs between 23.01.01 and 25.05.01 (See Chapter 2, 2.2 for further details).

3.3.2.2 Quantification of levels of cortisol

Levels of cortisol were measured in all urine samples using the EIA as described in Chapter 2 and corrected for urine dilution using the modified Jaffe end-point assay (Burtis & Ashwood, 2001; Chapter 2, section 2.3.2). Samples were diluted 1:256 to 1:512 as necessary and run in duplicate (see Chapter 2, 2.3) and corrected for creatinine concentrations following the procedure outlined (see Chapter 2, 2.3).

Table 3.1

Details of spider monkeys present during the visitor impact study.

ID	Date of birth	Age at start of study	Sex	Dam	Sire
Ric	18.12.93	8 yrs 1 mths	Male	Bru	Unknown
Mar*	1970[¥]	31 yrs	Female	Unknown	Unknown
Chr	30.09.88	12 yrs 3 mths	Female	Mar*	Art
Mil	05.11.90	10 yrs 2 mths	Female	Bla	Art
Zum	06.12.93	7 yrs 1 mth	Female	Del	Fre
Fay	22.02.94	6 yrs 11 mths	Female	Mar*	Fre
Sul	30.10.99	1 yr 2 mths	Male	Mar	Ric
Bog	09.02.00	11 mths	Male	Zum	Ric
Dor	24.03.00	10 mths	Male	Fay	Ric

* wild caught

[¥] estimate

3.3.2.3 Visitor study

The physiological impact of visitors on the monkeys was investigated by assessing the levels of urinary cortisol. Samples were collected during the FMD outbreak when no visitors were in the zoological park and throughout the year when visitor density fluctuated widely. This study used the total number of visitors in the zoo and related this to the concentration of urinary cortisol in the sample collected the next morning to account for a lag in the excretion of urinary cortisol (Bahr, et al., 2000; Whitten, et al., 1998). I selected samples using the Chester Zoo records and diary notes from the urine file. Samples were only included when I was confident that no other physical or social stressful events were occurring. I avoided any samples collected in the three days following a social or physical stressor and samples that preceded a known social conflict between animals as such events have been shown to impact cortisol levels in this group of spider monkeys (Chapter 5). In addition, a study of marmosets revealed that cortisol levels increase prior to the outbreak of serious conflict between animals (T. E. Smith & French, 1997a). Cortisol

values were only used on days when there were data points from two or more animals. The values from all animals were averaged on each day to provide one data point per time sampling interval.

It must be noted that for the period of seven months before the FMD outbreak and throughout the zoo closure, the breeding male had been separated from the rest of the group for animal management reasons. Although isolated from the rest of the group he still had full visual and also limited physical contact with the females and juveniles. He was reintroduced back into the group three weeks after the zoological park reopened. To ensure that this separation was not itself a source of stress we used a matched paired t-test to compare the mean urinary cortisol values for all members of the group during two periods when the breeding male was separated and after he had been reintroduced [t (5) = -.017, p = 0.987, Ric in: M = 2.13 ug cortisol/ ml per mg creatinine, SE = 0.40; Ric out: M = 2.14 ug cortisol/ ml per mg creatinine, SE = 0.29]. This indicated that the separation event did not confound the study.

3.3.3 Data analysis

The data presented here are derived from 179 urine samples (which resulted in 77 data points) collected across 77 days of urine collection. For details regarding contribution to the study see Chapter 2 (Table 2.4). On average we collected 35 urine samples from each monkey (range of 13 to 51 samples per monkey). I required at least two samples from two different monkeys on a given day to assess whether absolute visitor numbers were associated with urinary cortisol.

To determine whether there was a relationship between visitor number and cortisol we performed a Spearman's rank correlation, because the presence of 10 data points with a value of 0 led to a skewed distribution as revealed by a significant Kolmogorov-Smirnov test (Z = 1.62, N = 77, p <0.01). To explore the impact of visitors on HPA function we used a repeated measures one-factor analysis of variance (ANOVA) to compare cortisol levels across four categories of visitor numbers. These were no visitors (0), low (1-999), medium (1000-6999) and high (>7000) and were based on archived visitor data on the total number of visitors through the gate for a given day. When relevant, I assumed a two-tailed distribution and adopted an alpha level of 0.05 for all statistical tests.

3.4 Results

Mean values for all samples illustrated a trend for increasing cortisol values with higher numbers of visitors (Figure 3.1) and a positive correlation between urinary cortisol and number of visitors ($r_s = 0.43$, $p < 0.001$, N = 77, Figure 3.2) was identified. A repeated measures ANOVA was then used to compare cortisol levels from the five subjects across four visitor categories. We corrected for sphericity problems using Huynh-Feldt correction as recommended by Keppel (1993). We identified a non-significant result [F (3, 12) = 2.57, p = .156). However, further investigation of the data revealed that the cortisol levels in one subject (Fay) showed a conflicting trend with levels of cortisol decreasing with increasing visitor number categories. When the data from this individual subject were excluded from the ANOVA, a significant difference in cortisol levels was observed across the four visitor categories [(F (3, 9) = 10.82, p = 0.002].

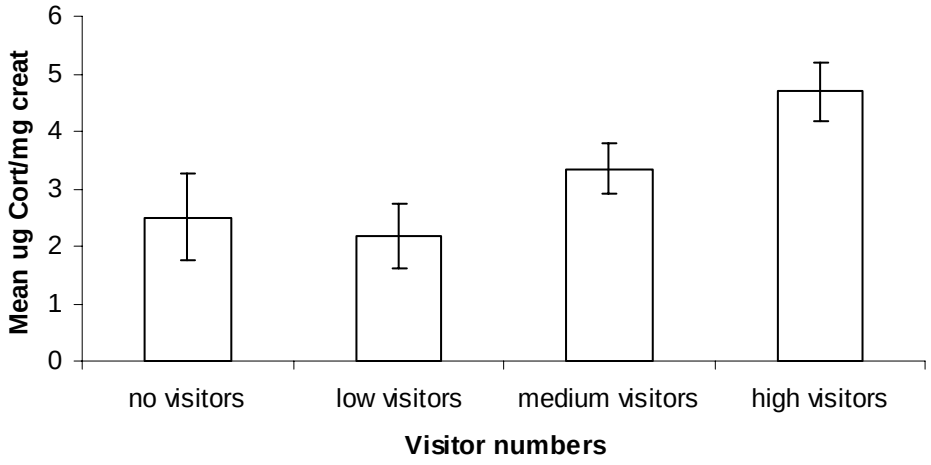

Figure 3.1 Mean values of cortisol for the visitor categories of no visitors (0); low visitors (1-999); medium visitors (1000-6999) and high visitors (>7000). Vertical lines depict the standard error of the means.

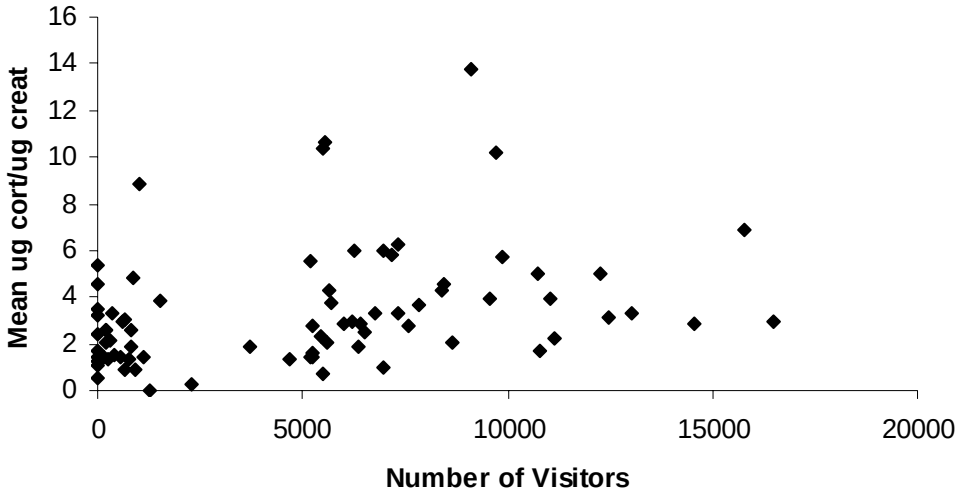

Figure 3.2 Levels of urinary cortisol are positively correlated with the number of visitors (p<0.001).

3.5 Discussion

The aim of this study was to increase the understanding of zoo visitor impact on spider monkeys by incorporating a physiological measure. My study supports previous behavioural research that visitors can have a meaningful impact on primates in zoos (Chamove, et al., 1988; S. Cook & Hosey, 1995; Fa, 1989; Hediger, 1969; Hosey, 2000). I found that as absolute visitor numbers increased, urinary cortisol increased, which suggests that visitors had an impact on spider monkey physiology. The latter relationship was positive and it was not likely attributable to a Type I error as alpha equalled 0.00009. However, the slope of the data points was not steep, and levels were still relatively low as compared to other known social stressors (see Chapter 5), suggesting that although increasing visitor numbers at Chester Zoo were associated with an increase in cortisol, large numbers of visitors are not a highly stressful experience for these spider monkeys. One intervening variable that potentially precludes a more dramatic HPA response to the impact of visitors is the enclosure design. A captive animal will be more able to cope with a potentially negative stimulus, such as exposure to zoo visitors, if it is allowed to respond with active avoidance or escape responses (Carlstead, 1996). The study animals had the

choice to hide from visual contact with visitors as the enclosure provided a variety of locations where the spider monkeys could be concealed from view, including tunnels, thick vegetation and grassy mounds (see Chapter 2, Figures 2.4 to 2.7). Previous research has demonstrated that animals which control their environment, experience less stress than animals with no control (Weiss, 1968).

The majority of subjects, four out of five, demonstrated the statistically significant trend of rising cortisol levels with increasing visitor numbers. One subject however showed the opposite trend. One possible explanation is that visitors did not affect this subject, and inter animal variability is well documented (Boccia, et al., 1995; Moberg, 1985, 2000; Mormede, et al., 2007; see Chapter 1, section 1.4.7). Alternatively, the HPA response of this subject to visitors might have been masked by additional factors since cortisol is modified by factors in addition to stress such as reproductive status, age and social dynamics (Abbott, et al., 2003; M. R. Clarke, et al., 1996; Gust, et al., 2000; Saltzman, et al., 1998). Being a retrospective study with no specific period of observations it is also possible that potential stressors such as aggressive incidents or reproductive events which were not recorded or observed could have been missed. These potential factors could therefore not be controlled for.

While the study suggests that visitors do have an impact on cortisol levels in the majority of adult spider monkeys the data for all monkeys were probably influenced by some of these potentially confounding variables since they were out of my control. Where possible their impact was accounted for, for example the separation of the male for management reasons was assessed.

This study only looked at the physiological aspect of a stress response which on its own maybe be difficult to interpret (G. Mason & Mendl, 1993). For example, one possible factor on cortisol levels is the effect of locomotion (Coleman, et al., 1998; Mormede, et al., 2007). Rates of activity are known to correlate with cortisol in marmosets (T. E. Smith, et al., 1998), although there appears to be some species differences (Mormede, et al., 2007). As a number of studies show a link between visitor numbers and increased activity (Hosey & Druck, 1987; Hosey, et al., 2009b; Mitchell, Herring, et al., 1992; Wells, 2005) it is possible that any increases in cortisol in the current study may be due to increases of locomotion associated with the higher visitor numbers rather than a stress response to the high visitor numbers. Therefore the interpretation of these results would have been aided by the collection of behavioural data in a more integrative approach (Dawkins, 2004). Based purely on

anecdotal evidence from observations during this study no obvious changes in activity patterns occurred across varying visitor numbers. In addition, when I assessed the impact of the introduction of a new male on this same group of spider monkeys that involved cortisol and behavioural measures, there was no correlation between locomotion and cortisol levels (Chapter 6). Subtle changes in rates of scratching or where animals position themselves in the enclosure might however reveal more of a response (Carder & Semple, 2008; Maestripieri, et al., 1992).

The assessment of visitor numbers was based on overall visitor attendance at Chester zoo, and therefore some discrepancy between the assessment of visitor numbers and actual numbers at the spider monkey enclosure was possible. A count of visitors at the spider monkey enclosure may have provided a more accurate assessment. However, the position of their enclosure near the main entrance is likely to be correlated with actual numbers at the enclosure. There are additional factors that may influence the impact of visitors beyond that of sheer numbers. The behaviour of visitors, (Birke, 2002; Mitchell, Tromborg, et al., 1992), their viewing position (Chamove, et al., 1988) and the installation of visual screens (Blaney & Wells, 2004) have all been shown to mediate the effect visitors have on non-human primates and could also be taken into account.

An elevated GC level in itself does not necessarily indicate a negative effect on an animal's welfare and moderate increases in GC are associated with optimized vigilance (Wiepkema & Koolhaas, 1993), enhanced learning, increased alertness and exploration (Chamove & Anderson, 1989). The ability for an individual to respond to short-term stressors could even be seen as beneficial, as the stimulation of the HPA axis would incite positive arousal (Chamove & Moodie, 1990). Long-term exposure to a stressor (chronic stress), however can have serious implications for an animal's welfare (Moberg, 2000). There are therefore inherent difficulties when interpreting physiological changes and a multidisciplinary approach including behavioural and various physiological data has been recommended to assess welfare of captive animals (G. Mason & Mendl, 1993).

To conclude I quantified levels of urinary cortisol in captive spider monkeys in response to varying visitor numbers. Levels of urinary cortisol increased with rising visitor number suggesting that visitors had a potential negative impact on the monkeys. Although the increases in cortisol were not high when compared to known stressful events they still could, if sustained over long periods, be a concern for the

welfare of spider monkeys in zoological parks. That a response was found in a zoo enclosure that is large and complex enough to allow the animals to choose to be out of view of zoo visitors is interesting. These finding have implications for other zoo exhibits where spider monkeys do not have such a choice and should be considered in the design of new enclosures and management practices.

CHAPTER 4

PATTERNS OF INJURY IN ZOO-HOUSED SPIDER MONKEYS: A PROBLEM WITH MALES?

4.1 Introduction

There is variation in the social organisation of non-human primates, ranging from solitary to pair living to multi-male/multi-female communities, the latter commonly characterised by female philopatry and male dispersal (Kappeler & van Schaik, 2002). In addition, most group-living species are characterised by a high degree of group cohesion. Some species (e.g., chimpanzees, *Pan troglodytes*, and spider monkeys, *Ateles* spp) live in groups that are characterised by a high degree of fission-fusion dynamics in which individuals travel in small fluid subgroups or parties that change in membership throughout each day (Chapman, Wrangham, & Chapman, 1995; McFarland Symington, 1990). Fission-fusion dynamics are thought to have evolved as a means to reduce intragroup competition over spatially and temporally distributed fruit (Aureli, et al., 2008; Chapman, Fedigan, Fedigan, & Chapman, 1989; McFarland Symington, 1987, 1988, 1990). In addition, fissioning is used as a way to reduce the escalation of aggression in wild spider monkeys (Aureli & Schaffner, unpublished data). A second feature of chimpanzee and spider monkey social organisation is male philopatry and female dispersal, in which males remain in their natal group and females leave to join new groups upon reaching sexual maturity (McFarland Symington, 1990).

In the wild the spider monkeys average group range size is 278 Ha (Di Fiore & Campbell, 2007), with communities varying in size from 15 to 56 individuals (Shimooka, et al., 2008). The demographics of communities are highly varied both across communities and species. There are reports of 1 to 15 adult males, 5 to 18 adult females, 0 to 7 sub-adult males, 0 to 7 sub-adult females and 1 to 10 juveniles (Shimooka, et al., 2008). Male-male social relationships are reported to be the most affiliative as they spend more time together and groom each other more than any other adult age-sex combination (Aureli & Schaffner, 2008). The reported pattern of aggression in wild spider monkeys involves males targeting females most frequently.

Males target females by chasing them to the ground although they are very rarely physically attacked (Campbell, 2003; Fedigan & Baxter, 1984; McFarland Symington, 1987; Slater, Schaffner, & Aureli, 2008; van Roosmalen & Klein, 1988). However, male-male aggression was unreported until recently in wild communities (Aureli & Schaffner, 2008; van Roosmalen & Klein, 1988), but two recent reports indicate that male-male aggression can be severe and in some cases lethal among intra-community males (Campbell, 2006b; Valero, Schaffner, Vick, Aureli, & Ramos-Fernandez, 2006). Aggression between females however appears to be rare in wild populations (van Roosmalen & Klein, 1988), although there are reports of long-term resident females targeting newer immigrant females (Asensio, Korstjens, Schaffner, & Aureli, 2008).

I observed two different incidents of male-male aggression between the adult male and two juvenile males at Chester Zoo. One case resulted in the death of a juvenile male that was related to the adult male. As a consequence, it was realised that very little information was available about aggressive behaviour in zoo-housed spider monkeys. However, a variety of factors are known to influence aggression in other captive primates, including the presence of human visitors in zoological parks, reduction in their living space, changes in group composition, variation in reproductive and social status and a lack of control over their physical and social environment (Honess & Marin, 2006a; Hosey, 2005; Morgan & Tromborg, 2007).

The aim of this chapter was to develop a questionnaire to investigate the prevalence of aggression in zoo-housed spider monkeys and determine whether there was a relationship between group composition and patterns of aggression. In particular information about the direction, intensity and context of any reported aggressive behaviour among the monkeys was requested. In addition, to investigate the influence of the physical environment on the occurrence and pattern of the aggression, there was a follow up request for information about enclosure dimensions. Based on the patterning of aggression reported from field studies (see above), three sets of predictions were made. Firstly, adult males would be the most frequent actors of aggression and that adult females would be the most frequent targets of minor aggression. Secondly, adult males would be the actors of severe and lethal aggression and that the juvenile males would be the targets. Finally, it was predicted that females would be the least frequent actors of aggression.

4.2 Methods

4.2.1 Procedure

I developed my questionnaire to obtain information on incidents of aggression in zoo housed spider monkeys based on an earlier study that used a similar tool for exploring patterns of aggression in captive lion tamarins (Inglett, et al., 1989). The primary aim was to collate accurate information in a form that would reduce subjectivity and over generalisation and that could then be analysed. Using the International Species Information System (ISIS, 2008) I identified a total of 55 zoological parks world-wide that maintained social groups of at least three adult spider monkeys with a mixed sex composition. A questionnaire was distributed in English in March 2002 by email to the appropriate curators, along with a covering letter, which requested information on any recorded aggressive events that had occurred in the previous five years (Appendix A). In addition, I requested that any available ARKS (Animal record keeping system) or suitable zoo records, which are maintained by the keepers responsible for the spider monkeys, be returned with the questionnaire. The first question requested information about the species of spider monkey. At the time of drafting the questionnaire four species were housed regularly in zoological parks, including *A. belzebuth, A. fusciceps, A. geoffroyi,* and *A. paniscus*. However, *A. fusciceps* has recently been reclassified and is now recognised as *A. geoffroyi rufiventris* (Rylands, et al., 2000; see Chapter 2). The questions focused on the frequency, context, direction and intensity of aggressive events; the age and sex of the individuals involved; the group composition at the time of aggressive events and the patterning of spider monkey aggression relative to other species that were housed at the same zoological park.

Age classifications were categorised with adults over six years old (van Roosmalen & Klein, 1988), sub-adults from four to six years, juveniles from two to four years and infants under two years. The resulting age-sex categories were as follows: adult males, adult females, sub-adult males, sub-adult females, juvenile males and juvenile females. The aggressive incidents were classified into three categories of different intensities based on the descriptions provided: “minor”, which included either no observed injuries or superficial injuries; “severe”, which included single, or multiple wounds that required veterinary treatment; and “lethal”, when the

individual was killed outright or where the injuries were so serious they necessitated that the individual be euthanized. Thirteen zoological parks also provided information about the size of the spider monkey enclosures. I requested the information as a follow up to the initial questionnaire as social behaviour, including aggressive interactions can be influenced by the area of the enclosure (Caws & Aureli, 2003; Hosey, 2005; Judge & de Waal, 1997; Kummer & Kurt, 1965) and the number of individuals in the social group. This additional information was then used to assess area as a potential factor in the prevalence of aggression.

4.2.2 Analyses

To ascertain whether the distribution of the age/sex class of the actors or the targets of aggression differed from a chance distribution chi-square tests were used for goodness-of-fit. The expected frequencies of aggression were weighted by correcting the expected values by the proportion of individuals in each age/sex class in the population to reflect the opportunity for aggression. To minimise the potential for violating the underlying assumption of independence for chi square tests, individual spider monkeys were counted only once for each category of aggression. This was a conservative approach as it had the effect of underestimating the incidences of aggression by individuals who show repetitive aggressive behaviour and so restricting the sample size. When sample sizes were less than six per cell the Yates correction was applied (Schwiegert, 1994). To assess whether there was a relationship between the density of animals in the enclosure and aggression, the densities of 13 enclosures were calculated based on the modal number of animals in the group over the course of the study and the area of the combined indoor and outdoor enclosure. Pearson's correlations were used to test for the relationship between density and mean number of aggressive events. The conventional alpha level of 0.05 was adopted for all tests.

4.3 Results

Thirty two of the 55 questionnaires were returned (58%), although data from eight zoological parks (14.5%) had to be omitted because the required group

composition had changed and no longer met the minimum requirements for my study, or insufficient information was provided about the overall group composition. Therefore, information regarding 26 different groups of spider monkeys from 24 zoological parks was provided. There were seven groups (26.9%) in which no aggression was reported, which were subsequently referred to as non-aggressive groups, and 19 (73.1%) groups in which aggression was reported, which were subsequently referred to as aggressive groups. The modal overall composition for the data set (n = 26) was one adult male and two adult females. The modal composition for non-aggressive groups (n = 7) was one adult male and one adult female, while the modal composition for aggressive groups (n = 19) was one adult male, two adult females and one non-adult male. Infants were not included in the analyses as they are not involved in any aggressive interactions.

Four species were represented in the survey data *A. belzebuth* (n = 7), *A. paniscus* (n = 3), *A. geoffroyi* (n = 6) and *A. fusciceps* (n = 10). The species *A. fusciceps* were kept in the largest overall group sizes (including infants) and *A. geoffroyi* were ascribed the highest number of aggressive incidents (Table 4.1). However, chi-square tests did not identify a significant difference in the number of aggressive events among the different species for the actors ($\chi^2(3) = 1.98$; $P > 0.05$) or targets of aggression ($\chi^2(3) = 4.07$; $P > 0.05$), therefore the entire dataset, regardless of species, was pooled and analysed together.

Although information from 143 incidents was obtained, for the investigation into actors and targets, only data where the identities of either the targeted individuals (targets) or the actors of aggression (actors) were known were analysed. This yielded 56 events for the actors and 127 events for the targets of aggression.

4.3.1 Frequency of aggression

Aggression was observed in the majority of spider monkey groups (see Table 4.2), with tension between males identified as the most common context of aggression. The majority of the respondents considered the nature of spider monkey aggression to be different to that of other primates housed within their zoological park. Specifically, they reported aggressive bouts were less frequent, usually occurred without any obvious signs of previous tension and often resulted in more

Table 4.1

Composition of groups by species/subspecies for overall modal group size, modal number of adults and non-adults, modal number of males and females and total number of aggressive incidents.

Species/sub species	*A. belzebuth*	*A. fusciceps robustus*	*A. g. geoffroyi*	*A. paniscus*	Total
Number of groups	7	10	6	3	26
Modal group size	6	7	4	4	4
(adult/non-adult)	(3/0)	(6/0)	(4/0)	(2/1)	(3/0)
(male/female)	(1/2)	(1/5)	(1/3)	(1/2)	(1/2)
Total number of incidents	5	46	91	1	143

severe injuries to the target animals. They also reported that aggression often occurred when younger males approached breeding age.

Using the criteria described above, 127 cases of aggression were recorded, with 93 cases (73.2%) of minor aggression, 28 cases (22.0%) of severe aggression and six cases (4.7%) of lethal aggression reported.

4.3.2 Actors and intensity of aggression

Overall, I found that males were much more likely to be aggressors than females (χ^2 (1) = 26.18; P <0.001). When the data were examined further by age class, it was found that adult males were the most frequent actors representing 37 cases (66.1%), followed by sub-adult males who accounted for 11 cases (19.6%) and adult females who accounted for 8 cases (14.3%). There were no instances of non-adult females or juvenile males as actors of aggression (see Table 4.3 and 4.4). Analysis of all the intensities of aggression revealed an overall significant difference for actors (χ^2 (5) = 32.31; P <0.001). The finding was driven by the adult males who

Table 4.2

Summary of response to the questionnaire showing number of responses for each question and proportion of answers.

Question	Number of Respondents	Number and proportion for each response			
1. Was aggression observed	26	Yes (19) 0.73	No (7) 0.27		
2. Context of aggression	16	Tension (8) 0.5	Introduction (3) 0.19	Bullying (1) 0.06	Feeding (4) 0.25
3. Aggression different to other primates	9	Yes (7) 0.78	No (1) 0.11	Not known (1) 0.11	

were more likely to be aggressors than juvenile males (χ^2 (1) = 12.41; P <0.001), adult females (χ^2(1) = 19.09; P <0.001), sub-adult females (χ^2 (1) = 13.16; P <0.001) and juvenile females (χ^2 (1) = 14.48; P <0.001), and by sub-adult males who were more likely to be aggressors than juvenile males (χ^2 (1) = 10.35; P <0.001), adult females (χ^2(1) = 17.03; P <0.001), sub-adult females (χ^2 (1) = 11.10; P <0.001) and juvenile females (χ^2 (1) = 12.42; P <0.001). There was an overall difference in the distribution for actors of minor aggression from the expected distribution across the six age/sex class categories (χ^2 (5) = 19.21; P <0.005). Age/sex pairwise comparisons yielded significant differences from the expected distribution as sub-adult males were more likely to be the actors of mild aggression than adult males (χ^2 (1) = 11.35; P <0.001), juvenile males (χ^2 (1) = 8.27; P <0.005), adult females (χ^2 (1) = 10.88; P <0.001), sub-adult females (χ^2 (1) = 9.35; P <0.005) and juvenile females (χ^2 (1) = 9.60; P <0.005). When severe aggression was examined there was also

Table 4.3

Summary of reported aggressive incidents towards adult male, sub-adult male and juvenile male spider monkeys as classified by age, sex and intensity.

Actors of aggression		Targets of aggression											
		Adult male				Sub-adult male				Juvenile male			
	N	minor	severe	lethal	**Total**	minor	Severe	lethal	**total**	Minor	severe	lethal	**total**
Adult male	6	1	3	1	**5**	2	0	0	**2**	6	3	2	**11**
Sub-adult male	3	0	1	1	**2**	0	0	1	**1**	1	0	0	**1**
Juvenile male	0	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
Adult female	3	1	0	0	**1**	3	0	0	**3**	0	0	0	**0**
Non-adult female	0	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
Group		9	2	0	**11**	1	0	0	**1**	1	1	0	**2**
Unknown		5	1	0	**6**	3	2	0	**5**	10	5	1	**16**
Total		16	7	2	**25**	9	2	1	**12**	18	9	3	**20**
N		8	5	2		7	1	1		9	6	3	

Total = total number of incidents

N = number of individuals involved

Table 4.4

Summary of reported aggressive incidents towards adult female and non-adult female spider monkeys as classified by age, sex and intensity.

Actors of aggression		Targets of aggression							
		Adult female				Non-adult female			
	N	minor	severe	lethal	total	minor	severe	lethal	Total
Adult male	4	11	2	0	**13**	5	1	0	**6**
Sub-adult male	3	6	0	0	**6**	1	0	0	**1**
Juvenile male	0	0	0	0	**0**	0	0	0	**0**
Adult female	3	3	1	0	**4**	0	0	0	**0**
Non-adult female	0	0	0	0	**0**	0	0	0	**0**
Group		6	1	0	**7**	2	1	0	**3**
Unknown		15	1	0	**16**	1	3	0	**4**
Total		41	5	0	**46**	9	5	0	**14**
N		15	5	0		5	1	0	

Total = total number of incidents

N = number of individuals involved

more of a difference in the distribution of actors across the six age/sex classes than expected (χ^2 (5) =15.20; P <0.01). The significant difference from the expected distribution was driven by the adult males as they were more likely than expected to target severe aggression compared to sub- adult males (χ^2 (1) = 6.38; P <0.025), juvenile males (χ^2 (1) = 7.58; P <0.01), adult females (χ^2 (1) = 7.16; P <0.01), sub-adult females (χ^2 (1) = 8; P <0.005) and juvenile males (χ^2 (1) = 7.42; P <0.01). Finally, the distribution for actors of lethal aggression differed from an expected distribution across the four age/sex classes (χ^2 (5) = 19.12; P <0.001). The difference was due to adult males being more likely than expected to be responsible for lethal aggression compared to sub-adult males (χ^2 (1) = 7.26; P <0.01), juvenile males (χ^2 (1) = 7.68; P <0.01),adult females (χ^2 (1) = 8.48; P <0.005) sub-adult females (χ^2 (1) = 8.38; P <0.005) and juvenile females (χ^2 (1) = 7.36; P <0.01).

4.3.3 Targets and intensity of aggression

Overall, males were more likely than expected to be the targets of aggression, regardless of intensity (χ^2 (1) = 18.29; P <0.001). Adult females were the targets of aggression in 46 cases (36.2%) of the aggressive bouts reported. Adult males were the targets of aggression in 25 cases (19.7%), juvenile males in 20 of the cases (15.7%), non-adult females in 14 of the cases (11.0%) and sub-adult males were targets of aggression in 12 of the cases (9.4%) (see Table 4.3 and 4.4). The distribution across age/ sex categories for all aggression differed from the expected distribution (χ^2 (3) = 81.19; P <0.001), however the significant effect was attributable to juvenile males who were more likely to be targeted than expected compared to adult males (χ^2 (1) = 81.27; P <0.001), sub-adult males (χ^2 (1) = 84.50; P <0.001), adult females (χ^2 (1) = 90.31; P <0.001), sub-adult females (χ^2 (1) = 90.13; P <0.001) and sub-adult females (χ^2 (1) = 81.60; P <0.001).

When the distribution, according to the different intensities, was examined more variation in the pattern was identified. The distribution across age/sex categories for minor aggression differed from the expected distribution (χ^2 (5) = 49.70; P <0.001). The deviation from the expected distribution was due to juvenile males receiving more mild aggression than expected compared to adult males (χ^2 (1) = 28.80; P <0.001), sub-adult males (χ^2 (1) = 31.86; P <0.001), adult females (χ^2 (1) = 31.84; P <0.001), sub-adult females (χ^2 (1) = 39.69; P <0.001) and juvenile females (χ^2 (1) = 29.56; P <0.001). The distribution for targets of severe aggression also differed from the expected distribution across the four age/sex classes of individuals (χ^2 (5) = 32.24; P <0.001). The deviation also differed due to juvenile males receiving more severe aggression than expected compared to adult males (χ^2 (1) = 37.00; P <0.001), sub-adult males (χ^2 (1) = 36.76; P <0.001), adult females (χ^2 (1) = 39.73; P <0.001), sub-adult females (χ^2 (1) = 36.75; P <0.001) and juvenile females (χ^2 (1) = 39.00; P <0.001). Finally, the distribution for targets of lethal aggression also differed from expected (χ^2 (5) = 40.99; P <0.001). Juvenile males were more likely than expected to be targets of lethal aggression than adult males (χ^2 (1) = 30.58; P <0.001), sub-adult males (χ^2 (1) = 31.29; P <0.001), adult females (χ^2 (1) = 34.86; P <0.001), sub-adult females (χ^2 (1) = 32.92; P <0.001) and juvenile females (χ^2 (1) = 32.33; P <0.001).

4.3.4 Animal density

No correlation between the density of spider monkeys and total mean number of aggressive events ($r = 0.08$; N = 127; $p = 0.81$) was found. Furthermore, no significant correlations were found between density and mean number of minor aggressive events ($r = 0.08$; N = 93; $p = 0.79$), severe aggressive events ($r = 0.03$; N = 28; $p = 0.93$) and lethal aggressive events ($r = -0.13$; N = 6; $p = 0.67$).

4.3.5 Context of aggression

Of the 143 cases of aggression a detailed explanation was provided by the respondents for 54 (37.8%) of the incidents, and these were separated into five distinct contexts. The most frequent context for aggression was tension among males within the same group, defined as a long-standing situation between two males in which they had repeated conflicts that were not resolved, and was reported by 10 zoological parks and accounted for 50% of the incidents described. The context of introduction, when animals were reintroduced to the group after a period of separation or when new animals were introduced to the group, was reported by seven zoological parks accounting for 25.9% of the incidents. 'Bullying' when the whole group chased and harassed an individual, was reported in four zoological parks accounting for 20.4% of the incidents described by zoo keepers. The least frequent context of aggression was public feeding reported by one zoological park accounting for 3.7% of the incidents. General aggression in the group, aggression by males and aggression by females were also reported, but not enough detail was provided to indicate a specific context.

4.4 Discussion

The use of questionnaires as a tool to quantify animal behaviour has been widely used in both applied and theoretical contexts but rely on the fundamental assumptions that the person caring for the animal has access to valid information about the animal's typical behaviour, and that this information can be extracted in a form that is reasonably reliable and accurate (Gosling, Kwan, & John, 2003; Hsu & Serpell, 2003). The data collected in this study were based on animal records that

zoo staff members make on a daily basis, rather than notes from diary entries or memory which were deemed less reliable. To further improve the quality of the data only zoos that were accredited to ISIS, which are committed to keep records to a high standard, were used. Although no pilot study was carried out prior to the questionnaire being distributed its design was based on a previous study into aggression in zoo housed lion tamarins (Inglett, et al., 1989). However, a prototype may have been useful in refining and modifying the questions which may have assisted in the interpretation of the data provided.

The questionnaire provided information about 143 aggressive incidents which were used to evaluate the predictions. Four species of spider monkey were represented in the questionnaire data and although there was variation in the total numbers of incidences of aggression across the different species analyses revealed no significant difference. This allowed the dataset to be pooled. These differences are likely to be attributable to differences in demographics and group size, and that most of the aggressive incidents occurred in a small proportion of the groups. The first prediction was that adult males would be the most frequent actors of minor aggression and that adult females would be the most frequent targets of minor aggression. Although adult males were responsible for the majority of the reported minor aggression, with 25 of the 40 individual cases, and adult females received 41 of the 93 cases of minor aggression, when the proportion of age and sex classes were considered as part of the overall population the results did not support either of these predictions. It was the sub-adult males who were more frequently the actors and juvenile males more frequently the targets of minor aggression than expected. This differs from reports in the wild, which indicate female-directed male aggression is the most frequently reported aggression (Campbell, 2003; Fedigan & Baxter, 1984; Slater, et al., 2008). Reports of minor aggression between males are virtually absent from wild populations. For example, van Roosmalen and Klein's (1988) review does not include any reports of male-male aggression.

The second prediction, that adult males would be the most frequent actors of severe and lethal aggression and that the juvenile males would be the most frequent targets was supported. Adult males were overwhelmingly responsible for severe and lethal aggression. Males committed 9 of the 11 cases of severe aggression. In addition, juvenile males were more likely than expected to be the targets of severe and lethal aggression. The proportion of aggressive incidents that resulted in severe

injuries (22.0%) is high compared to data from several long-term studies of captive primates in which no severe aggression was reported (Bernstein, Williams, & Ramsay, 1983; Fuentes, Malone, Sanz, Matheson, & Vaughan, 2002; Ren, et al., 1991; Thierry, 1985; Zucker, 1994), although it is likely that minor injuries were under reported as they could only be based on direct observations. Males were responsible for all six cases of lethal aggression.

Although lethal aggression has been reported in captive chimpanzees (*Pan troglodytes*) (de Waal, 1986a) and captive golden lion tamarins (*Leontopithecus rosalia*) (Inglett, et al., 1989), generally reports of lethal aggression are rare. For example, in a two year study of a captive group of chimpanzees involving 219 conflicts only three incidents resulted in observable injuries (0.14%) (Fuentes, et al., 2002), in a four month study of two groups of captive golden monkeys (*Rhinopithecus roxellanae roxellanae*), where 130 agonistic encounters were observed, no injurious aggression was recorded (Ren, et al., 1991). In a much larger study of macaques 1322 aggressive interactions were recorded in rhesus macaques (*Macaca mulatta*), 570 in a group of long-tailed macaques (*M. fascicularis*) and 682 in a group of tonkean macaques (*M. tonkeana*) over a 12 month period, and only 56 (2.2%) agonistic interactions with bites were recorded (Thierry, 1985). In a group of free ranging patas monkeys (*Erythrocebus patas*) 1353 agonistic interactions were recorded over seven months, of which 6.13% were bites (Zucker, 1994), and in a year long study of agonistic behavior in large groups of captive rhesus macaques (*M. mulatta*), stumptail macaques (*M. arctoides*), pigtail macaques (*M. nemestrina*), Sulawesi black crested macaques (*M. nigra*) and sooty managabeys (*Cercocebus atys*) adult males participated least of any age-class in any agonistic encounters and seldom involved in any forms of contact aggression (Bernstein, et al., 1983). Importantly no lethal aggression was reported in any of these studies.

Field studies however have revealed cases of lethal intragroup aggression illustrating that aggression does occur in the wild. Itani's (1982) review of intragroup lethal aggression revealed killing occurred in 13 species of nine genera. He separated them into two categories including infanticide, which accounted for the vast number of instances, and rare events of killing among adults. Recent studies reveal that lethal intragroup aggression also occurs in white handed gibbons (*Hylobates lar*) (Palombit, 1993), chimpanzees (*Pan troglodytes*) (Fawcett & Muhumuza, 2000; Nishida, 1996; Watts, 2004; Watts, Muller, Amsler, Mbabazi, & Mitani, 2006),

capuchin monkeys (*Cebus capucinus*) (Gros-Louis, Perry, & Manson, 2003) and spider monkeys (*Ateles* spp) (Campbell, 2006b; Valero, et al., 2006). Thus, there appears to be something unusual about the intensity of spider monkey aggression that occurs in zoos, with such a high percentage of aggressive incidents which involve individuals receiving substantial and potentially life-threatening or lethal injuries. There may also be a possible difference between the four species with general aggression much more prevalent in the *A. g. geoffroyi* and to a lesser extent *A. g. rufiventris.* However, this may be explained by the fact they were generally kept in larger groups and so had an increased potential for aggression between conspecifics

Finally, I predicted that females would be the least frequent actors of aggression. The data supported this prediction as only eight incidents of aggression were attributable to adult females, and all but one incident was categorised as minor. Non-adult females were never the actors of aggression. This is not surprising as very low rates of female-female aggression are reported in wild populations of spider monkeys (Fedigan & Baxter, 1984; van Roosmalen & Klein, 1988), with the exception that resident females do target aggression toward new immigrant females (Asensio, et al., 2008). However, the analogous context, the introduction of a new female into an existing group, only occurred once in my study groups and did not result in any female-female aggression.

One explanation for the pattern of aggression identified is that male-male aggression in zoo-housed spider monkeys may reflect natural behaviour that occurs in wild communities. Intragroup aggression between males has been reported in the wild and recent studies from two field sites (Campbell, 2006b; Valero, et al., 2006) and indicates that male-male aggression, albeit rarely observed, may be more serious than the more commonly reported male-female aggression (Fedigan & Baxter, 1984; van Roosmalen & Klein, 1988). Valero et al. (2006) reported on a single lethal attack, while Campbell (2006b) reported on three separate attacks, which all resulted in serious injuries and in two cases had a presumed lethal result. Campbell reported all three attacks involved a coalition of resident adult males attacking a single non-adult resident male. Such intragroup lethal aggression is surprising given that males are philopatric, are likely to be related and have the strongest social bonds. For example, males affiliate with each other more frequently (Ahumada, 1992) and travel in all-male subgroups more than other age/sex classes (Shimooka, 2005). In addition, males defend their territory during inter-group encounters (L. L. Klein, 1974;

McFarland Symington, 1990), and make joint raids into neighbouring communities (Aureli, Schaffner, Verpooten, Slater, & Ramos-Fernandez, 2006). There is also negligible sexual dimorphism in spider monkeys, which has been linked to low levels of male-male competition (Dixson, 1998; Fedigan & Baxter, 1984).

Intermale relationships among primates in intra- and intergroup competition are highly variable across species (Kappeler, 1999) but are generally shaped by female distribution and competition for fertilizations. This has typically been characterised by competition, intolerance and clear dominance relations with agonistic interactions common among males in primates (Kappeler & van Schaik, 2002). Spider monkeys are unusual in that they do not demonstrate a clear dominance hierarchy and instead form strong bonds and demonstrating well developed coalitionary behaviour (Aureli & Schaffner, 2008), a characteristic that seems to be linked with male philopatry. The recent discoveries of severe and lethal aggression however also suggest a degree of complexity in this relationship.

Further support for the finding that males are responsible for the majority of aggression in zoos was provided by questionnaire respondents. Zoo keepers indicated that the most frequent context of aggression was 'tension' between males. These descriptions indicated that this context represented a long-standing situation between two males in which they had repeated conflicts that were not resolved. In addition, this tension was particularly noted to occur between non-adult and adult males. The remaining contexts of aggression, feeding by zoo visitors and reintroducing group members or introducing new group members are well-established sources of short-lived aggression in a variety of primate taxa (Honess & Marin, 2006b; Hosey, 2005). There was also a consensus by the respondents that aggression in spider monkeys differs to that of other primate species. In particular, spider monkey aggression was characterised as infrequent, involving non-adult males more often and was more severe than what the keepers observed in other primate species.

Several explanations might account for the higher than expected levels of aggression between males reported in zoological parks. Firstly, one proximate mechanism in promoting better relationships among males is the need to cooperate in defending their home territory (Aureli, et al., 2006). In a zoo setting, the absence of rivals may reduce the value of male social relationships (Aureli, Cords, & Van Schaik, 2002) and lead to a greater degree of male intolerance. A second explanation may lie in the management practices of zoos. Males are regularly relocated between

zoo groups (Pierre Gay personal communication), which does not correspond to the pattern of immigration in wild groups where females disperse (Ahumada, 1992; Fedigan & Baxter, 1984; L. L. Klein & Klein, 1971; McFarland Symington, 1990; van Roosmalen & Klein, 1988). Such practices inevitably lead to more unrelated males housed together than would occur in the wild, which could be further a source of tension (see Eisenberg & Kuehn, 1966). The practice of relocating males and not females (Durlot & Gay, 1998; Newland, 1999) could influence rate and intensity of aggression, although this factor was not explicitly examined in the questionnaire. While there may be management difficulties keeping groups of spider monkeys in their 'natural' group structure its feasibility should be investigated, particularly as maintaining animals in abnormal social groupings is a known source of stress (Honess & Marin, 2006b; Hosey, 2005; Morgan & Tromborg, 2007). A policy that would include the transfer of females, and not males, between zoos would not be unprecedented as this is the general management policy for chimpanzees in zoological parks (Carlson, 2006; Fulk, 2000).

Finally, it is well-established that captive environments may alter the behaviour of individuals relative to their wild counterparts (Hosey, 2005; Kummer & Kurt, 1965). These include variations in enclosure size and complexity (Carlstead, et al., 1999; Van Keulen-Kromhout, 1978) and management routines (Bassett & Buchanan-Smith, 2007). Specifically, Hosey (2005) highlighted that for zoo-housed primates the presence of visitors, restricted space and management practices all impact behaviour, and social factors are likely to interact with these variables. For example, the social system of a given species, which can vary in the extent of group cohesion and mating patterns (van Schaik & van Hoof, 1983), could be influenced under captive conditions. Therefore, individuals that live in social systems characterised by a high degree of fission-fusion dynamics could be particularly affected given that in the wild they have the option of leaving a subgroup to reduce conflict (Aureli & Schaffner, 2007; Rebecchini in prep). This is relevant because although most of the zoo housed groups were small with a modal group structure of one male and one female, the groups which had more males had more incidents of aggression. While a positive relationship between the density of the spider monkeys in zoos and the number of aggressive episodes was not found this could be explained by the coping strategies adopted by other primates when restricted to a confined space (Aureli & de Waal, 1997; Caws & Aureli, 2003; Judge & de Waal, 1997).

However, small enclosures do reduce opportunities for fissioning, which may influence the intensity of aggression in zoo-housed spider monkeys.

The information from this questionnaire highlights a phenomenon that has not been previously reported in zoo-housed spider monkeys although has been recently discovered in the wild. The unusually high prevalence of serious aggression reported may be linked to the current social management practices in zoos. Collectively, the lack of rival males, housing unrelated and unfamiliar males together and having no means to simulate fission are factors that may lead to patterns of male aggression that exceed what would be expected by chance alone. These factors also indicate the importance of providing suitable housing and husbandry practices for spider monkeys. Specifically, larger and more complex enclosures that allow opportunities for individuals to temporarily separate themselves from the larger social group, in order to simulate fission events in the wild, may reduce the frequency and severity of aggression (Caws, Wehnelt, & Aureli, 2008; Wehnelt, Bird, & Lenihan, 2006). The unnatural social and physical environment in which spider monkeys tend to be kept in zoos may exacerbate the propensity for male against male aggression in the wild. Therefore, managing zoo populations of spider monkeys should also entail the relocation of females rather than males.

CHAPTER 5

THE IMPACT OF SOCIAL EVENTS ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS OF ZOO-HOUSED SPIDER MONKEYS (*ATELES GEOFFROYI RUFIVENTRIS)*

5.1 Introduction

Primates are highly intelligent sentient beings that display a complex behavioural repertoire and require a stimulating environment (McCann, et al., 2007). Their cognitive sophistication makes them particularly susceptible to psychological stress arising from a variety of social and environmental factors, not only through actual stressors but also in anticipation of stress (Sapolsky, 2003, 2004). It is therefore essential that the appropriate conditions that satisfy their behavioural and social needs are met when in captivity to ensure their wellbeing (Boissy, Manteuffel, et al., 2007; Chapter 1).

Reducing stress levels is one way in which welfare of captive animals can be improved (Boissy, Manteuffel, et al., 2007; Chapter 1). Although the stress response to a variety of laboratory procedures is well documented in several primate species, it is still a relatively new area of research (Honess & Marin, 2006a). To date, such studies include investigations into environmental (Crockett, et al., 2000) and social factors (Abbott, et al., 2003) and reproductive status (Setchell, et al., 2008).

A number of factors have previously been identified that can affect GC levels in primates. These include species variation, individual variation, age, season, reproductive status, social status, aggression, social support, reproductive condition, male immigration, risk of infanticide, rank instability, predation, seasonal changes and the availability of resources (Abbott, et al., 2003; Anestis, Bribiescas, & Hasselschwert, 2006; Lane, 2006; Setchell, et al., 2008). In addition, there are many potential sources of stress for primates housed in captive settings (Morgan & Tromborg, 2007) (see Chapter 1). These can include routine husbandry events, presence of care staff, anticipation of feeding, sound, threat of predation, resource scarcity, a non stimulating environment, being housed alone, changes in composition and social dominance rank (for review see Honess & Marin, 2006a). One of the main

sources of stress in a captive environment is loss of the opportunity for species-specific behaviours for which any animal has a behavioural need (Chapter 1).

A zoo environment offers specific conditions which distinguish it from other forms of captivity (see Chapter 1, section 1.5; Honess, et al., 2005; Hosey, 2005). Many aspects of an animal's life history, such as feeding and reproduction, are managed and are therefore beyond the control of the animals. Being confined can also reduce the ability of an individual to respond to aversive situations with appropriate escape or avoidance responses, which for social primates in particular can be significant (Hosey, 2005). These stressors can be in the form of proximity to predators, competing conspecifics, unfamiliar sounds, keeper interactions and the presence of visitors (See Chapter 3; Carlstead & Shepherdson, 2000; Hosey, 2000).

Although the impact of husbandry practices and social relationships on the HPA axis has been assessed in a number of primate species (Honess & Marin, 2006a), there have been only a handful of studies looking specifically at stressors within a zoo environment (Shepherdson, et al., 2004), and none previously reported in zoo-housed spider monkeys. Previous research into social stress in primates has predominately examined it from the perspective of dominance hierarchy relationships, which is important in many species and considered to be a major source of psychological stress (Abbott, et al., 2003; Cavigelli, Dubovick, Levash, Jolly, & Pitts, 2003; Engh, et al., 2006b). Modifying group membership can also be a significant source of social stress with potential to activate the HPA axis (Honess & Marin, 2006b). I first examined the various factors that influence the stress response and how they can be influenced within a zoo environment.

5.2 Environmental factors influencing the stress response

A considerable body of research has been carried out in laboratories on the assessment of housing conditions and husbandry practices on physiological measures of stress in non human primates (Clarke, Harrison, & Didier, 1996; Crockett, et al., 2000; Mendoza, Capitanio, & Mason, 2000; Whitten, Stavisky, Aureli, & Russell, 1998). For example, enclosure size and its structural complexity (Honess, et al., 2005) has been identified as a potential major source of stress in primates with links to the performance of abnormal behaviours, infant mortality, aggression and growth

rates (Morgan & Tromborg, 2007), although studies have shown mixed results. For example, one study increasing enclosure size for great apes found little or no effect on behaviour (S. F. Wilson, 1982), while a study into orang-utans (*Pongo pygmaeus*) found increasing size and usable space did predict changes in behaviour (Perkins, 1992). A decrease in abnormal behaviours was found with increases in enclosure size in rhesus macaques (*Macaca mulatta*) (Paulk, Dienske, & Ribbens, 1977), although no decrease was found in a study into long tailed macaques (*M. fascicularis*) (Crockett, et al., 1995) or pig tailed macaques (*M. nemestrina*) (Crockett, et al., 2000). However, it is the quality of space that is just as important as the size.

The provision of a complex and stimulating environment is now widely accepted as important in the general health and wellbeing of animals kept in captivity (Honess & Marin, 2006b). This concept of environmental enrichment, in particular, recognises the importance of allowing animals the opportunity to perform species-specific behaviours. Such conditions allow for greater control over their environment, which is important since a lack of control over the environment has been identified as potentially the greatest source of stress for animals in captivity (Sambrook & Buchanan-Smith, 1997). Furthermore, increased behavioural options allow animals to respond to adverse environmental conditions by managing confinement related stress. This has been demonstrated by reductions in GC levels in capuchins (*Cebus apella*) following environmental enrichment (Boinski, et al., 1999). However, while moving animals to more complex environments may, in the long term, be beneficial, in the short term a novel environment may cause a significant stress response (Hennessys, Mendoza, Mason, & Moberg, 1995; T. E. Smith, et al., 1998).

An environment that provides opportunities for animals to retreat from other conspecifics and reduces proximity to humans or potential predators is beneficial for certain species. In particular, providing places for retreat or to hide is important when unfamiliar animals are introduced into new groups, which is often unavoidable as part of captive husbandry and can often be a cause of aggression (Doyle, et al., 2008; Morgan & Tromborg, 2007). In a zoo environment the presence of visitors can be a significant factor (Davey, 2007; Hosey, 2000; see Chapter 3), although the effect can differ across different taxa and species and is dependent on the animals flight distance to humans (Hosey, 2008). The provision of areas of retreat can be beneficial. For example, through the provision of a camouflage barrier in front of

visitors, reduced aggression and stereotypic behaviour was observed in gorillas (*Gorilla gorilla*) (Blaney & Wells, 2004). Proximity to animal carers is also a potential source of stress, (Hosey, 2008) although if relationships are positive then close contact can produce friendly interactions and can be a source of enrichment. The handling of animals however, can be a substantial source of stress, even for animals that have been trained (Bassett, et al., 2003; Honess & Marin, 2006a). Other potential sources of stress include inappropriate environmental variables such as temperature, light, substrate and odour (Morgan & Tromborg, 2007), husbandry routines (Bassett & Buchanan-Smith, 2007) and feeding and foraging opportunities (Morgan & Tromborg, 2007).

5.3 Social factors influencing the stress response

Primates are highly social and intelligent animals normally living in groups (Fuentes, 2007; Kappeler & van Schaik, 2002; Silk, 2007). Group living offers a variety of benefits including improved detection and protection from predators, an increased likelihood of finding a resource as well as defending it from others, an increased chance of finding a potential mate, the transfer of information such as the location of resources, and also the facilitation of alloparental care (Bernstein, 2007; van Schaik, 1989). However, it also has the negative consequences of increased direct competition over resources potentially leading to conflicts and aggression (Bernstein, 2007; van Schaik, 1989). While there is a general interest within a group in keeping the costs of competition low, and maintaining a cohesive network of social bonds and mutual dependencies (de Waal, 1986b), the potential for social stress from this competition is a constant possibility (Kikusui, et al., 2006). To overcome this, many primate groupings are characterised by dominance hierarchies, which are generally established through aggressive conflicts and then maintained through reliable signals of submission and dominance (Bernstein, 2007; de Waal, 1986b; Preuschoft & van Schaik, 2000). These highly ritualised contexts are designed for maximum benefit but minimum risk with the general principle that an animal consistently and without resistance abandons their place when approached by a more dominant group member (Kummer, 1971a). Knowledge of previous fight outcomes can be used to predict the outcome of the next fight, although appropriate

action will still depend on evaluation of the incentive and the assessment of the determination of the opponent to contest that incentive using aggression (Bernstein, 2007). Although such behavioural mechanisms have been adopted to prevent aggressive escalation, conflicts of interest may be unavoidable for group living animals (Aureli, et al., 2002).

Social factors both alleviate and exacerbate the physiological response to stressful stimuli, making primates a good model to investigate links between the social environment and the physiological stress response (T. E. Smith & French, 1997a). Living in social groups offers the benefit of companionship (Kikusui, et al., 2006), and this is demonstrated by a high stress response when individuals are separated (Noble, McKinney Jr, Mohr, & Moran, 1976). Social animals also show a better recovery from aversive experiences when they are together (Mendoza, Coe, Lowe, & Levine, 1978).

5.3.1 Social buffering

Social stressors are known to be particularly effective in stimulating the HPA axis (Mendoza, et al., 2000). Complex social affiliations can, however, provide social support protecting the animals from the consequences of stress (Levine, 2000). For example, being accompanied by a familiar group member has benefits in reducing the effect of social separation stress (Kikusui, et al., 2006; T. E. Smith & French, 1997b). This is known as social buffering or social support (Cohen & Wills, 1985).

Given the various types of social organisation displayed by primates, different responses to various social stressors occur in different species, depending on their relationship with their social buffering partner (Mendoza, et al., 2000). For example, this is illustrated by the differences in stress response during exposure to novelty and separation of monogamous titi monkeys (*Callicebus moloch*) and group living squirrel monkeys (*Saimiri sciureus*) (Hennessys, et al., 1995). During isolation the GC response to novelty was significantly more sensitive in titi monkeys than in squirrel monkeys indicating the high value of social partners for this species.

The cues responsible for social buffering will also depend on the species and on how they communicate social information to their conspecifics, but can be tactile, olfactory, vocal or visual (Kikusui, et al., 2006). In primates the importance of contact behaviour has been demonstrated in the rearing of rhesus monkeys (*M.*

mulatta) (Winslow, Noble, Lyons, Sterk, & Insel, 2003) and vocal buffering has been shown to reduce urine cortisol levels in isolated marmosets (*C. kuhlii*) (Rukstalis & French, 2005).

5.3.2 Aggression

Conflict is inevitable among individuals in a social group and several studies have examined the environmental and social factors that regulate conflict and relationships in order to maintain group cohesion (Aureli, et al., 2002; Honess & Marin, 2006b). Aggression is a high risk strategy and primates often rely on non-contact ritualised aggression or dominance displays to reduce risks associated with less predictable more overt forms of aggression (Bernstein, 2007; Preuschoft & van Schaik, 2000).

5.3.2.1 Dominance

Researchers have examined social factors that impact on the stress response in non-human primates with considerable focus on the context of dominance and social status (Abbott, et al., 2003; Creel, 2001). Studies indicate that the position in the hierarchy, whether dominant or subordinate, is generally maintained through a specific form of aggression: re-directed aggression (Sapolsky, 1990), and the unpredictable nature of this aggression is thought to contribute to the stress response (Sapolsky, 2004). Dominance interactions have physiological consequences on the HPA axis response, although there is no simple relationship and these interactions can vary across different species (Creel, 2001; Engh, et al., 2006; Ostner, Heistermann, & Schülke, 2008; Setchell, et al., 2008). As a consequence of these profound species differences in their social relationships, there is considerable variation in the ways in which social factors modulate the stress response (T. E. Smith, et al., 1998), some of which are indicated below.

Callitrichids who live in family groups with a handful of breeding individuals, who are normally the parents of the other group members, showed a tendency for lower basal cortisol levels in subordinate females, e.g. Wied's marmosets *(C. kuhlii)* (T. E. Smith & French, 1997b). Squirrel monkeys (*Saimiri sciureus*) who live in large multi-male/ multi-female groups show approximately equal basal cortisol levels in dominant and subordinate females (Saltzman, Mendoza,

& Mason, 1991) and lower levels in dominant males (Manogue, 1975), whereas olive baboons (*P. anubis),* which also live in multi-male/ multi-female groups, have higher levels of GCs in subordinate males than dominant males (Sapolsky, 1982). Higher GC levels are found in high ranking dominant males than in subordinate males in Japanese macaques (*M. fuscata*) (L. Barrett, Gaynor, & Henzi, 2002) and ring-tailed lemurs (*L. catta*) (Cavigelli, 1999).

5.3.2.2 Aggression within and between the sexes

Aggression between individuals of the same sex is related to dominance and can be explained through reproductive competition (Honess & Marin, 2006a). Due to the seasonal availability of resources many primate species are seasonal breeders (Dixson, 1998). An increase in aggression during the mating season is widely reported across a range of primates in the wild (Ostner, et al., 2008), although this has not always been replicated in captivity (Honess & Marin, 2006a). This rise in aggression has also been associated with an increase in levels of testosterone in males such as rhesus macaques (Herndon, Bein, Nordmeyer, & Turner, 1996), although this may be as a result of increased intrasexual aggression and not because of it (Cavigelli & Pereira, 2000).

Aggression between the sexes has been linked with a male reproductive strategy and intersexual selection, and may be associated with intensifying the social bonds that are required for reproductive success (Eaton, Modahl, & Johnson, 1981). Alternatively, as aggressive bouts are seldom associated with copulation it has been proposed that female-directed male aggression is nothing more than a mechanism for spacing and asserting dominance (Bercovitch, Sladky, Roy, & Goy, 1987). Another theory is that it is a means of sexual coercion, when force is used to increase the chances of a successful mating, or decrease the chances of a successful mating with another male (Clutton-Brock & Parker, 1995; Smuts & Smuts, 1993). Although this has been reported in relatively few species of primate, e.g. Japanese macaques (G. M. Barrett, et al., 2002) and orang-utans (*Pongo pygmaeus*) (Manson, 2007) it does occur where social organisation is characterised by fission-fusion dynamics and female dispersion (Slater, et al., 2008) and is normally associated with a behavioural stress response in the targeted females (e.g. Campbell, 2003).

Although it is generally believed that male primates are more aggressive than females (Reinhardt, 1987), this is not always the case. For example, work on captive

rhesus macaques revealed that aggression was more an individual character trait than dependent on sex or rank (Reinhardt, 1987). There is also limited evidence for age related affects on aggression, although increases are related more to reproductive maturation and rank (Honess & Marin, 2006a).

5.3.3 Reproductive behaviour

The effect of reproductive behaviour on GC levels has not been well studied although they are likely to be related to dominance. In a multi-male group living primate dominant males normally have a higher reproductive success than subordinates, although female choice may not necessarily be correlated with dominance rank (Manson, 2007). In a study on wild Japanese macaques (*M. fuscata*), rates of aggression and copulatory behaviour were the same in dominant and subordinate males, although cortisol levels were significantly higher in dominant males indicating a cost (G. M. Barrett, et al., 2002). In addition, it is possible that females choosing to mate with lower ranking males incur a cost in the form of increased aggression from dominant males in the group (Smuts & Smuts, 1993) and the potential cost of inferior genes.

There are also a handful of studies in non human primates that suggest giving birth may be stressful for females. In zoo-housed gorillas (*Gorilla gorilla*) individual variation in postpartum stress responses occur and appear to be related to failure of maternal behaviour (Bahr, Pryce, Dobeli, & Martin, 1998). There is also evidence of an increased stress response in lactating females when compared to non lactating females in captive rhesus macaques, which may be related to a heightened perceived risk from the mothers for their infants (Maestripieri, Hoffman, Fulks, & Gerald, 2008). Overall the link between cortisol and reproduction is complex as reproductive hormones can modify cortisol levels in various ways (see Chapter 1, section 1.4.9).

5.3.4 Group formations and introductions

Changes in group composition, such as recruitment of new individuals, and loss of individuals through emigration represent periods of potential instability for group living animals. In wild chacma baboons (*Papio hamadryas ursinus*), significant rises in mean GC concentrations were observed following the

immigration of unfamiliar males compared to when no immigration occurred (Beehner, Bergman, Cheney, Seyfarth, & Whitten, 2005; Engh, et al., 2006). However, such rises appeared not to be the result of male instability itself, but more specific to the alpha male and to females with dependent young. In a group of yellow baboons (*P. cynocephalus*) the immigration of an aggressive male led to an increase in GC levels in the resident group, particularly in the females, as well as the new male (Alberts, Sapolsky, & Altmann, 1992).

In captivity the introduction of new individuals into a group of rhesus macaques caused high levels of aggression and severe injuries (see Honess & Marin, 2006b). In Wied's marmosets the reaction of the females in the group to the introduction of a stranger was dependent on the size of the group, with smaller groups showing less aggressive behaviour (Schaffner & French, 1997), although the extent to which either result corresponded to higher GC levels is not known.

Due to the social nature of primates, separation and solitary housing is a well established stressful event (Boccia, et al., 1995; Crockett, Bowers, Bowden, & Sackett, 1994). Involuntary social separations have a substantial impact on the HPA axis causing increases in cortisol levels (Mendoza, et al., 2000), although this is also dampened by the presence of a preferred partner (Gust, Gordon, & Hambright, 1993; T. E. Smith, et al., 1998). However, separation of group members in captive settings is sometimes unavoidable for management reasons.

The intensity of the stress response due to separation and introductions can be mediated by age and sex (Gust, Gordon, & Hambright, 1993). For example, the separation of infants and juveniles is known to induce a severe behavioural stress response in the infants (Boccia, et al., 1995; Terao, Hamano, & Koyama, 1995). In contrast, the separation of adult male rhesus macaques from a group initially resulted in no response in the females remaining in the group or in the separated males, however, following reintroduction back into the group after a long-term separation a significant stress response occurred in both males (Gust, Gordon, Hambright, & Wilson, 1993). Below, I examined how these various influences on stress response may affect spider monkeys within a zoo environment.

5.4 Spider monkey social dynamics

5.4.1 Spider monkey social organisation: its relevance to stressors in captivity

Spider monkeys live in multi-male/multi female communities, distinctive among monkey social organisation because this system is characterised by a high degree of fission-fusion dynamics in which members of the community frequently split and merge into fluid subgroups, so much so that members of a single community are rarely, if ever, altogether (Aureli & Schaffner, 2008; see Chapter 1). Therefore, housing spider monkeys in confined settings, which precludes the opportunity for expressing any fission-fusion dynamics, could serve as a potential primary stressor in a zoo environment. Not only can captivity restrict the opportunities for natural behaviour but it can also reduce opportunities for animals to retreat from potential sources of stress, such as visitors or conspecifics (Hosey, 2005; see Chapter 1).

5.4.2 The dynamics of aggression in spider monkeys

Studies into aggression in monkeys have primarily been carried out on species that are not characterised by high fission-fusion dynamics. There are no previous studies carried out on *Ateles* that examine the relationship between aggression and their GC response. Spider monkeys also differ from most of their Old World counterparts because there is no evidence of clear cut dominance relationships in spider monkeys (Aureli & Schaffner, 2008), a hallmark of the social lives of many Old World primates (Kappeler & van Schaik, 2002).

Spider monkeys also show low levels of affiliative behaviours such as grooming (Ahumada, 1992; Fedigan & Baxter, 1984; Schaffner & Aureli, 2005; Slater, et al., 2009). Such behaviours have been used in studies on primates in captivity as a means of a behavioural assessment of a stress response (Schaffner & Aureli, 2005), so this may make behavioural assessments more difficult in spider monkeys than other species. They do, however, have a suite of species-specific behaviours, which include embraces and pectoral sniffing, that may be used as a means of conflict management during potential periods of conflict immediately following an episode of separation (Aureli & Schaffner, 2007) and maybe useful as an alternative means of behavioural assessment. Scratching behaviour has also been

used in previous studies in other primates as a non invasive means of measuring psychosocial stress (Maestripieri, et al., 1992) and may also be relevant in spider monkeys.

In wild spider monkeys the most commonly reported aggression is directed by males towards females, although physical attacks are rare (Campbell, 2003; Campbell & Gibson, 2008; Fedigan & Baxter, 1984; McFarland Symington, 1987; Slater, et al., 2008). Such aggression is believed to be a form of sexual coercion when females are ovulating (Slater, et al., 2008). Rates of aggression between males, however, are rarely reported. Males form the strongest bonds within a spider monkey community and spend most of their time together (Ahumada, 1992; Aureli & Schaffner, 2008; Slater, et al., 2008). However, there have been two recent reports of lethal aggression in the wild by adult males towards younger males (Aureli & Schaffner, 2008; Campbell, 2006b; Valero, et al., 2006). Also, there have been observations that relationships between young and older males are uncertain with young males keeping a safe distance (Aureli & Schaffner, 2008; Vick, 2008). Indeed, as presented in Chapter 6, I found that in captive spider monkeys adult males were responsible for all cases of severe and lethal aggression.

Aggression between adult females is relatively rare (Fedigan & Baxter, 1984; Slater, et al., 2008) and relationships between unrelated females are reported to be of lower quality in spider monkeys (Aureli & Schaffner, 2008; Di Fiore & Campbell, 2007; Fedigan & Baxter, 1984; McFarland Symington, 1990). In fact, aggression in females is even low during periods of low food availability (Campbell & Gibson, 2008). Although there are no long term established dominance patterns between adult females, the older more established females have been reported to direct aggression towards newer immigrants who could be viewed as competing for resources (Asensio, et al., 2008; Chapman, et al., 1995; McFarland Symington, 1987). This could be explained by fission-fusion dynamics reducing scramble competition at the sub group level (Asensio, et al., 2008).

Competition for resources is believed to be the main antecedent of fission-fusion in spider monkeys – in particular for food (Aureli, et al., 2008; McFarland Symington, 1990). In a zoo environment where food is plentiful and therefore the proximate triggers for competition are reduced, aggression may be less frequent. However, when one of the primary mechanisms for reducing competition in spider

monkeys is removed (Aureli & Schaffner, 2008), i.e. the ability to fission from the group, it is possible that aggression could be exacerbated under captive conditions.

5.4.3 The social dynamics of reproductive behaviour

As previously reported, the most frequent aggression in spider monkeys is female-directed male aggression (Aureli & Schaffner, 2008). It has been proposed that such aggression is linked to the reproductive state of the female (Campbell, 2003; McFarland Symington, 1987) and may be part of a ritualized intimidation display (Fedigan & Baxter, 1984). This has been supported in a recent study by Slater et al (2008). The authors found that aggressive male-female interactions could be split in to two categories of physical aggression involving contact, and a prolonged chase, the later taking place overwhelmingly during periods when the female was ovulating.

In addition, spider monkeys have unusual courtship patterns. In the wild the pair will deliberately and secretly move away from other member of their community as a "consortship" and stay away for what could be minutes or even full days (Campbell & Gibson, 2008). Behaviour during consortship suggests that they are avoiding other group members. Both the male and female are actively vigilant and avoid vocalising with other group members (Campbell, 2006a). This pattern of behaviour is also observed in captive settings with the male and female often leaving the rest of the group and finding a sheltered area away from other group members and maintaining a high degree of vigilance during copulation (personal observation).

A zoo environment may not allow opportunities for consortship because there is not enough space for the pair to move away from the rest of the group. This could potentially be stressful for the male during mating events and could be reflected in an increase in cortisol levels at this time. However, the reason for the secrecy in the wild situation may be due to male competition for females. As this is not present in most zoo settings (ISIS, 2008), their need for secrecy may not be as essential for successful mating to take place, but may still constitute a significant source of stress for both male and females if it is not enabled.

Unlike old world primates, spider monkeys do not exhibit visual demonstration of reproductive status, such as swellings, and instead researchers have to rely mainly on behavioural clues. Due largely to the often secretive nature of copulations, sexual

behaviour in wild and even captive spider monkeys is rarely observed (Campbell & Gibson, 2008). Despite this, it has been suggested that ovulation is associated with a number of distinctive behaviours. They include copulation (Symington, 1987), place sniffing where the male sniffs the place where a female was sitting (L. L. Klein & Klein, 1971), clitoral stimulation by males and females (van Rooselmalen, 1985) and urine sniffing (Campbell, 2004). However, endocrinological data have shown that none of these behaviours are strictly associated with ovulation as they have been observed, albeit less frequently at other stages of the reproductive cycle (Campbell, 2004). It is likely that they are also used by the males as a means of gauging a female's reproductive condition; therefore care should be taken when using behaviour alone as a means of assessing reproductive status (Campbell & Gibson, 2008). Cycle lengths of captive and free-ranging spider monkeys fall consistently between 20 and 24 days, with menstrual bleeding present over 2-4 days. However, this is not always externally visible and therefore cannot be relied on as a visual indicator of female reproductive status (Campbell, et al., 2001).

5.4.4 The impact of social events on HPA activity in zoo-housed spider monkeys

The present study investigated the impact of social factors on GC levels in a zoo-housed group of spider monkeys using a non-invasive measure of stress: urinary cortisol. Although stress research is used in the assessment of husbandry practices and social relationships in a number of primate species (Honess & Marin, 2006a), there have been only a handful of studies looking at various stressors within a zoo environment (Shepherdson, et al., 2004), and none previously reported in spider monkeys. Earlier research into social stress in primates has predominately examined it from the perspective of dominance hierarchy relationships, which is important in many species and considered a major source of psychological stress (Abbott, et al., 2003; Cavigelli, Dubovick, Levash, Jolly, & Pitts, 2003; Engh, et al., 2006). However, spider monkeys are reputed to have low or no linear dominance hierarchies (Aureli & Schaffner, 2008) making them an interesting test case for assessing various social factors on their HPA axis responses. In addition, studies of primate aggression have been primarily carried out on species that are largely cohesive, such as macaques, squirrel monkeys, titi monkeys and callitrichids, whereas spider monkey social organisation is characterised by high fission-fusion dynamics (Aureli, et al.,

2008) and therefore the effects of various social factors may have different impacts on their HPA responses. Finally, no previous studies carried out on *Ateles* have examined the relationship between aggression and their GC response.

5.5 Aim

The aim of this study was to investigate the impact of social factors on GC levels in a zoo-housed group of spider monkeys using urinary cortisol as a non-invasive measure of stress. The particular categories of social events examined were aggression, reproduction and separation. Based on previous studies, it was believed that these particular events could be associated with an increase in cortisol in zoo-housed spider monkeys.

5.5.1 Predictions

Based on behaviour studies from the wild (Asensio, et al., 2008; Fedigan & Baxter, 1984; Slater, et al., 2008), and from a survey on aggression in spider monkeys in zoological parks (Chapter 4) I predicted the majority of minor aggression would be carried out by adult males towards receptive adult females when ovulating, or to a lesser extent by adult females to sub adult females as they approach maturity. Aggression between adult females would not be expected. Any cases of severe or lethal aggression would be expected by adult males towards sub adult males.

Regarding cortisol it would be expected that the largest increases would be associated with the most severe cases of aggression (Ostner, et al., 2008), with severe and lethal aggression associated with the largest increased levels of cortisol. It would also be expected that the response would be dependent on the individuals' role in the aggressive act with the targets of aggression experiencing the largest increase in urinary cortisol, followed by the actors of aggression and then the bystanders (Creel, 2001; T. E. Smith & French, 1997b). With respect to the timing of cortisol changes, I predicted that cortisol would be elevated the day following aggression and that the rate at which cortisol levels returned to pre-event levels would depend on the severity of the aggression.

Previous studies into reproductive events have shown increases in GCs associated with seasonality and increased competition by males for females (Manson, 2007). However, as spider monkeys are not seasonal breeders (Vick, 2008), and with only one adult male in the study group, competition for females would be at a minimum. Although minor, female-directed male aggression is reported in the wild (Slater et al., 2008; Fedigan & Baxter, 1984), it is unlikely that this would cause a pronounced stress response as it appears to be ritualised in spider monkeys. However, because the spider monkeys investigated in the present study are zoo-housed it may prove difficult for them to engage in species-specific secretive mating (Campbell & Gibson, 2008), therefore I predicted there would be increased GCs in the actors of sexual behaviour at the time of consortships. In addition, postpartum increases in GCs have also been seen in primates in captivity, which have been linked to maternal behaviour (Bahr, et al., 1998; Behringer, et al., 2009) and an increased sensitivity to a stress response from mothers with infants (Boccia, et al., 1995; Maestripieri, et al., 2008).

Severe stress responses have been associated with separations and reintroductions in a number of primate species in captivity (Brent, Kessel, & Barrera, 1997; Clarke, Harrison, & Didier, 1996; Honess & Marin, 2006a; Mendoza, et al., 2000). Therefore, I predicted that separating individuals from the social group would lead to an increase in cortisol levels in the separated individual and to a lesser extent in bystanders. In addition, I predicted that long-term separation would lead to higher cortisol levels than short term separations. Finally, I predicted that reintroduction of group members would also lead to increased cortisol responses for both targets of the separation and bystanders.

5.6 Method

5.6.1 Urine samples

Urinary cortisol was used as an index of stress because the collection of samples was non invasive, it fitted in well with the daily routine of the group and is a proven method of measuring GCs in primates (Chapter 2). Levels of urinary cortisol were quantified in a total of 2140 samples from six adults present during the study between February 2000 and March 2005 (see Table 5.1).

Table 5.1

The number of urine samples used from each individual for each event.

Individual	Number of samples per event			
	Aggression	Reproduction	Separation	Total
Chr	305	298	78	486
Mil	75	79	29	129
Fay	192	197	53	314
Mar	251	265	70	416
Ric	314	295	80	500
Zum	193	170	60	295
Total	1330	1304	370	2140

5.6.2 Event categories

For aggression I investigated the intensity, the role of the individual and the timing. Intensity included three levels: minor (aggression which included either no observed injuries or superficial injuries); severe (aggression which included single, or multiple wounds that required veterinary treatment) and lethal (aggression when the individual was killed outright or where the injuries were so serious they necessitated that the individual be euthanized). These levels could be characterised retrospectively following the event and did not rely on the aggression being observed. The role of the individual also included three levels: the target, the actor and bystanders. The role of actor and bystander could only be determined by direct observations of aggressive incidents either by me or by keeping staff which were subsequently recorded. Finally, I also examined the effect of time on aggressive events. This included: prior (samples seven days prior to the event); at (sample from the morning following the event); and post (samples from the following seven days after the event).

For reproduction I investigated the type of event, the role of the individual and the timing of the event. Reproduction included three events: ovulation (signalled on the day by (i) the presence of blood in urine or (ii) the male sniffing in the location of where a female was or had been sitting or (iii) the male handling and sniffing a female's clitoris); mating (observed copulation between a female and the

male) and birth (delivery of an infant following full term pregnancy). The role of the individual in each reproductive event included: the adult male; female target (adult female experiencing the event) and bystanders (other adult females). These events and roles were determined and recorded by myself or by keeping staff throughout the study period as and when they occurred. The sample collection protocol for assessment of reproductive events was identical to that for aggressive events.

For separation I investigated the effect of type of event, the role of the individual and the timing of the event. The type of event included: temporary separation (an individual was out of the group for < 24 hr); separation (an individual was out of the group for ≥ 24 hr) and reintroduction (an individual or individuals were reintroduced back into the group following separation). During separations the individual was kept in a section of the enclosure at the back of the exhibit in visual and potential tactile contact with the rest of the group (see Chapter 2, section 2.1.6). The role of the individual included only two levels: the separated individual and bystanders (individuals not separated from the group). Samples were analysed from the week before to the week after each event. Due to the delay in cortisol being excreted in the urine (see Chapter 1, section 1.4.10) each sample represented the cortisol value for the day prior to the day of collection.

5.6.3 Defrosted samples

During storage, a number of samples (763 samples collected between November 2003 and July 2005) were accidentally defrosted over an unknown period of time (as persons unknown unplugged the freezer housing the samples) before they could be assayed. Of these samples, 458 were required for the present study. Following the discovery of the defrosted event, samples were relabelled to reflect the thaw and then immediately frozen back to -20°C. Cortisol is a cholesterol based steroid hormone and is fairly robust to defrosting (Miki & Sudo, 1998), however due to the unknown time period of the defrosting its effect was investigated. To assess the potential for degradation of the cortisol and check for any potential interference from any other substances that may have formed during the defrosted period, a pool of the defrosted samples (Pool C) was taken and tested against the normal pool (Pool B) for specificity (see Chapter 2, section 3.2). Pool C was comprised of six samples from each adult and five samples from each sub adult in this social impacts study,

comprising a total of 45 samples. This equates to 24 samples from adult females, five from a sub adult female, six from an adult male and 10 from sub adult males. For details on Pool B see Chapter 2, section 3.2. To avoid confounding factors any lower level events which overlapped with any major events were not analysed.

5.6.4 Analyses

Factors affecting levels of urinary cortisol were investigated by using linear mixed models (LMM's). LMM's allow both fixed and random variables to be fitted to a model, while controlling for variation due to repeated measures of individuals (Tabachnik & Fidell, 2007). The best model was selected by using Akaike's information criteria (AIC). It compares the adequacy of several models, identifying the model that best explains the variance of the dependent variable as that with the lowest AIC value (Tabachnik & Fidell, 2007). This approach has been used previously in zoo based research on primates were sample size is limited (e.g. O. N. Fraser, et al., 2008). Maximum likelihood (ML) method was used with fixed variables, and restricted maximum likelihood methods (RELM's) were used with interactions of fixed variables. An alpha level of 0.05 was adopted for all statistical analysis. The cortisol level was entered as a continuous dependent variable, with identity as the random variable in the models.

The first analysis compared the data samples across types of social event to see whether there were any differences across the event categories (i.e. aggression, reproduction or separation). The subsequent analyses were performed on each type of social event. Post hoc tests used pairwise comparisons using Least Significant Difference. The timing categories for each event were the same as described previously for aggression.

5.7 Results

The standard curve for the normal and defrosted pool was parallel and positively correlated ($r = 0.93$, $n = 7$, $P = 0.02$) indicating cortisol was still present in a highly consistent pattern, but at lower concentrations than non-defrosted samples (Figure 5.1). A correction value of 6.21 was calculated based on the cortisol value of

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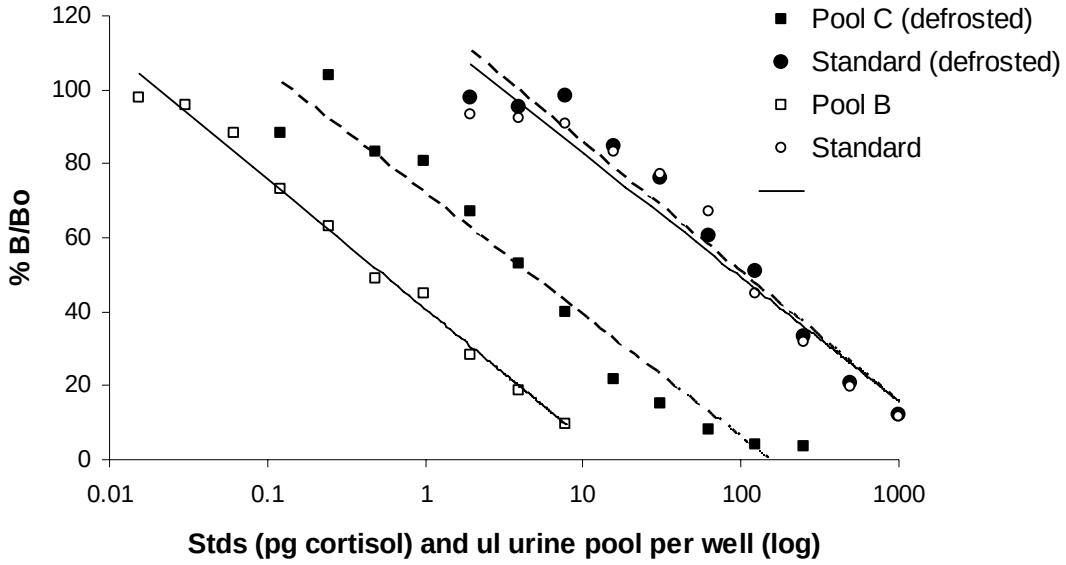

Figure 5.1 % B/Bo of serial dilutions of the Pool B (none defrosted) and Pool C (defrosted) and two cortisol standards to demonstrated parallelism.

pool C at the working dilution of 1:512 and applied to the defrosted samples. Applying such a conversion value has previously been carried out in a study in which a substance was found to consistently lower the measure of cortisol (Cross, Pines, & Rogers, 2004)

I examined the impact of all the stress factors initially in a model to determine whether there were differences in how the different social factors of aggression, reproduction or separation events impacted cortisol values in the spider monkeys. Overall mean values of cortisol for the three categories were 2.408 ± 0.31 SEM, 1.899 ± 0.31 and 1.498 ± 0.35 for aggression, reproduction and separation, respectively. Analyses with LMM's (ML) (see Table 5.2) revealed that, overall, aggression was responsible for the greatest degree of variance in cortisol levels [F (1, 3) = 10.191, P < .0001, AIC = 25940.544]

5.7.1 Aggression

A total of 60 aggressive incidents were recorded over the study period. When the actor of aggression could be identified the adult male (Ric) was largely responsible, accounting for 63% of minor cases and 44% of severe cases of

Table 5.2

LMM results for the three event categories.

Dependent variables	Continuous
Cortisol	
Fixed explanatory variables	
Event	1 = Aggression, 2 = Reproduction, 3 = Separation
Random variables	
ID	
Event	[F (1, 3) = 9.92, P < .00005, AIC = 22707.01]

aggression. These were carried out predominately towards adult females although incidents of female-female aggression were also recorded, in particular towards the youngest adult female. Finally, there were two cases of lethal aggression which were both carried out by Ric towards juvenile males.

A total of 1709 samples were used in the analysis of 60 incidents of aggression (see Table 5.3). When I examined the impact of different intensities of aggression on the cortisol values for actors, targets, and bystanders the week prior to the day of and the week following the aggressive events a three way interaction of type, role and time provided the best fitting model with the lowest AIC value. [F (1, 23) = 9.772, P < .0001, AIC = 9956.406]. The bystander values were calculated using the means of the individual animals so avoiding data pooling (see Table 5.4).

Each type of aggressive event was examined individually to determine where the differences across the three factors of the interaction lay. Cortisol levels for minor incidents (Figure 5.2A) showed very little variation across the role or time, with consistently low levels of cortisol throughout. Of the 29 incidences of minor aggression between adults where the actor was known, the adult male (Ric) was responsible for 20 events that were directed towards adult females. Of the 22 incidences between adults, where the target was known, the youngest adult female (Fay) was the recipient on 14 occasions.

For severe aggression (Figure 5.2B) a significant difference was found over time for actors [F (1, 2) = 3.264, P < .05, AIC = 778.694] and for targets [F (1, 2) =

Table 5.3

Number of events and number of samples used for each type of aggression category.

Role	Type			
	Minor	Severe	Lethal	Total
Actor	29	13	2	44
	(220)	(134)	(13)	(367)
Target	22	16	0	38
	(126)	(73)	(0)	(199)
Bystander	35	23	2	60
	(724)	(395)	(24)	(1143)
Total incidents	35	23	2	60
(samples)	(1070)	(602)	(37)	(1709)

6.909, $P < .05$, AIC = 500.228], with post hoc test showing a significant increase in cortisol levels from the week prior to the day of aggression before returning back to pre aggression levels in the week following for both roles. The adult male (Ric) was identified as the actor for 10 of the 23 incidences of severe aggression. Of the 16 aggressive events between adults, where the target was known, the youngest adult female (Fay) was the recipient on eight occasions.

For lethal aggression there was also a change in cortisol levels seen over time (Figure 5.2C). For the bystander role cortisol levels showed a significant difference over time [F (1, 2) = 6.928, $P < .01$, AIC = 142.531], with post hoc tests showing a significant increase on the day after the event against the week before. For actors levels of cortisol showed an increase in the week following the incident although this was not significant. The targets of the lethal aggression were juvenile males and no data were available for them as their data points were not available for the day of the aggression or the week after. The adult male was the actor for both cases of lethal aggression.

Table 5.4

Mean values and number of samples for every individual in the bystander role for each aggression and time category.

Category	Time	ID						Overall mean	Standard error
		Chr	Mil	Fay	Mar	Ric	Zum		
Minor	Pre	1.106 (115)	2.946 (19)	1.230 (37)	2.543 (91)	0.930 (55)	2.053 (63)	1.801 (380)	0.341
	At	1.361 (17)	1.908 (5)	1.117 (4)	1.746 (15)	1.079 (7)	1.716 (3)	1.488 (51)	0.143
	Post	1.776 (88)	3.259 (14)	1.003 (29)	2.379 (70)	1.629 (36)	1.702 (46)	1.958 (283)	0.316
Severe	Pre	1.713 (37)	3.833 (16)	5.593 (34)	3.672 (46)	1.451 (47)	2.142 (36)	3.067 (216)	0.649
	At	1.889 (6)		3.812 (4)	6.841 (7)	0.814 (6)	0.927 (4)	2.857 (27)	1.132
	Post	2.299 (28)	3.553 (7)	6.031 (18)	5.265 (25)	1.064 (28)	2.148 (22)	3.394 (128)	0.789
Lethal	Pre	1.285 (6)	3.263 (1)	0.803 (2)	2.935 (5)		1.526 (2)	1.963 (16)	0.481
	At	1.848 (1)		67.272 (1)				34.560 (2)	32.712
	Post				32.92 (1)		0.704 (1)	16.813 (2)	16.109

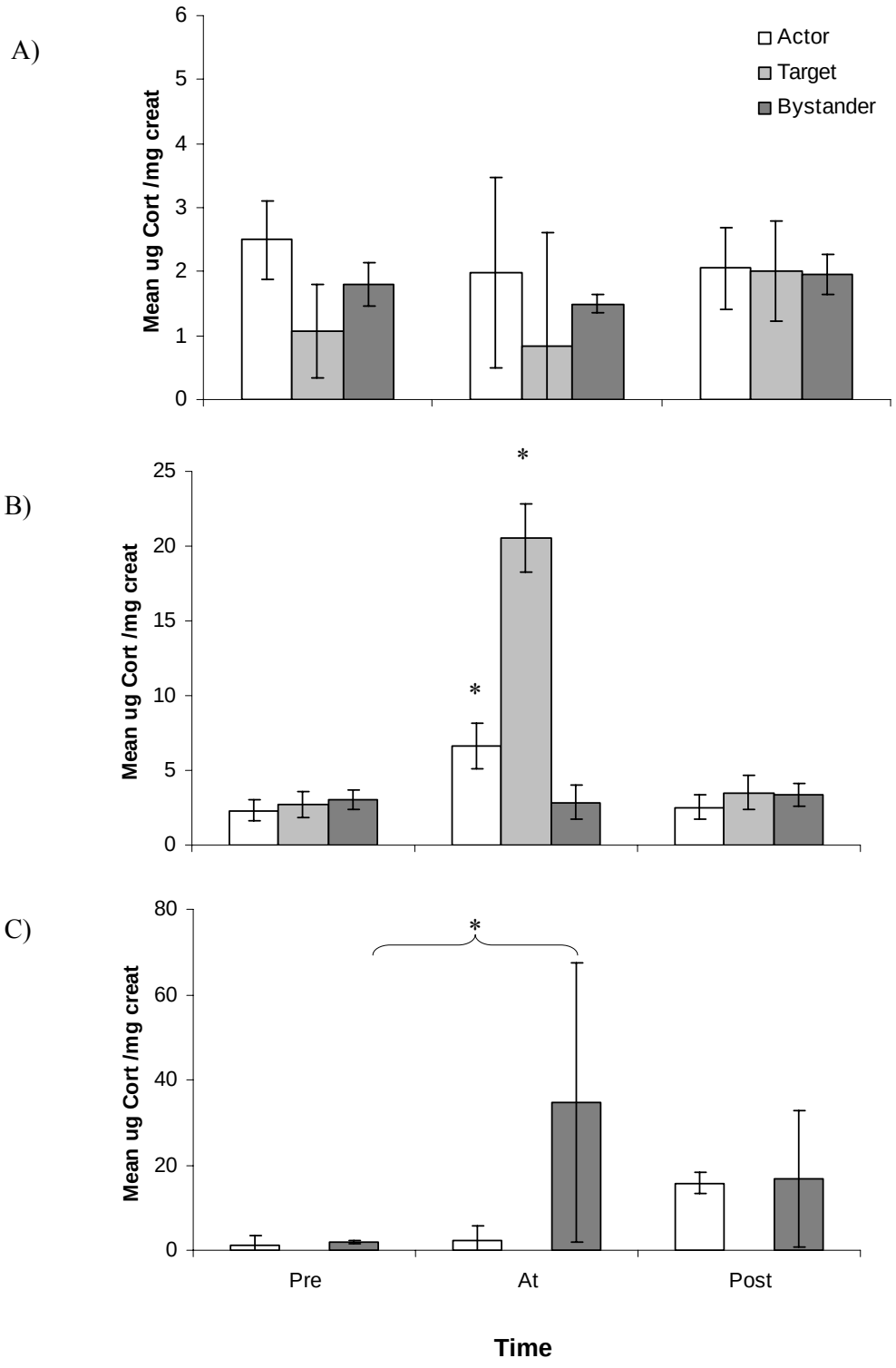

Figure 5.2 Mean values of cortisol over time for the actor, target and bystanders for the three aggressive events of A) minor; B) severe and C) lethal aggressive events. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

5.7.2 Reproduction

A total of 56 events and 1732 samples were used in the analyses of reproduction (see Table 5.5 for summary). When I examined the impact of different categories of reproduction on the cortisol values for the female, adult male, and other adult females the week prior to, the day of and the week following the reproductive events a three way interaction of type, role and time provided the best fitting model with the lowest AIC value. [F (1, 26) = 2.869, P < .0001, AIC = 9632.391]. For the other adult females category values were calculated using the means of the individual animals (see Table 5.6).

Table 5.5

Number of events and number of samples used for each type of reproduction category.

	Type			
	Ovulation	Mating	Birth	Total
Female	8	38	8	54
	(40)	(200)	(54)	(294)
Male	3	32	0	35
	(15)	(239)	(0)	(254)
Other females	9	39	8	56
	(168)	(811)	(205)	(1184)
Total incidents	9	39	8	56
(samples)	(223)	(1250)	(259)	(1732)

Each type of reproductive event was again analysed separately to determine the reasons for the differences. For ovulation (Figure 5.3A) there was again no effect of time with cortisol levels similar in the week prior, the day of and the week following the ovulation event, nor was there any difference in role. There were also no effects of time or role on levels of cortisol for mating events (Figure 5.3B).

Finally for the birth events (Figure 5.3C) cortisol levels changed significantly over time [F (1, 2) = 5.520, P < .01, AIC = 861.126], with post hoc tests showing

levels significantly higher in mothers than in the male or other adult females for the week prior to birth. There was also a trend for higher cortisol in mothers prior to the birth, which decreased following birth and returned to baseline levels in the week following. Cortisol levels for the other adult females also changed significantly over time [F (1, 2) = 6.108, P < .005, AIC = 1238.698], with post hoc tests showing levels significantly higher on the day of the event, compared to the week prior or the week following the event. Cortisol levels for the male did not fluctuate significantly across the three time periods.

Table 5.6

Mean values and number of samples for every individual in the other adult females role for each type of reproduction and time category.

Category	Time	ID					Overall mean	Standard error
		Chr	Mil	Fay	Mar	Zum		
Ovulation	Pre	0.940 (29)	1.606 (2)	6.751 (17)	1.864 (16)	1.308 (8)	2.494 (72)	1.075
	At	2.296 (3)		0.506 (3)	1.012 (2)	1.780 (3)	1.398 (11)	0.397
	Post	0.806 (13)	1.984 (2)	2.777 (15)	4.371 (6)	1.849 (7)	2.358 (43)	0.593
Mating	Pre	1.28 (113)	2.61 (53)	2.23 (98)	4.21 (99)	1.47 (45)	2.36 (408)	0.52
	At	1.184 (15)	3.172 (8)	0.692 (9)	1.087 (9)	0.985 (5)	1.424 (46)	0.445
	Post	0.986 (83)	2.646 (34)	1.214 (66)	1.732 (74)	1.784 (32)	1.673 (289)	0.287
Birth	Pre	0.804 (21)	1.436 (7)	2.623 (12)	1.416 (21)	1.772 (8)	1.610 (69)	0.297
	At	0.792 (3)		42.941 (2)	0.929 (3)	0.187 (1)	11.212 (9)	14.902
	Post	0.873 (17)	1.143 (6)	1.356 (10)	2.955 (22)	0.969 (11)	1.459 (66)	0.383

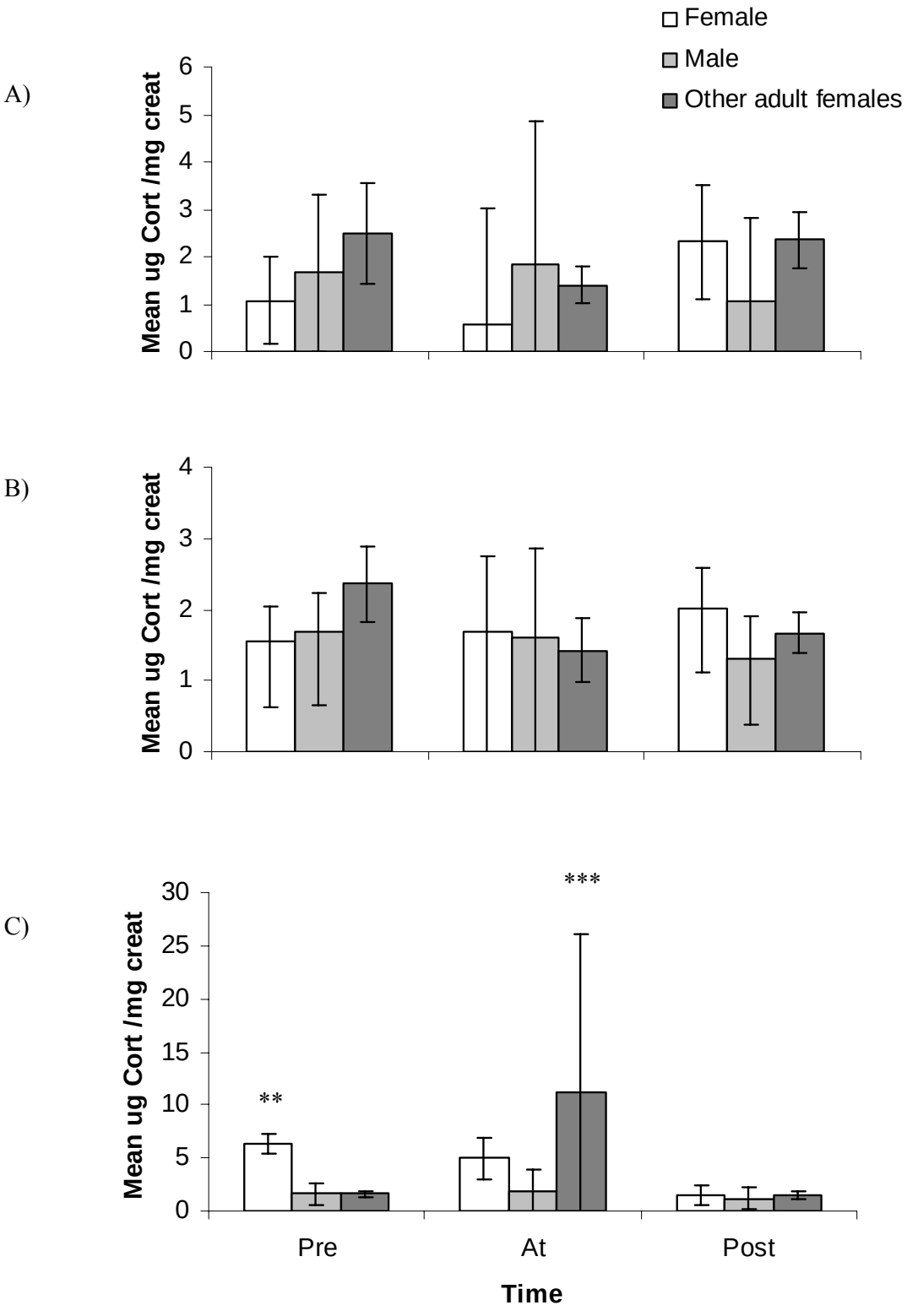

Figure 5.3 Mean values of cortisol over time for the female, male and other adult females for the three reproduction events of A) ovulation; B) matings and C) birth. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

5.7.3 Separation

A total of 15 events and 536 samples were used in the analyses of separation (see Table 5.7 for summary). When I examined the impact of the three different types of separation on the cortisol values for the separated/reintroduced individuals and the bystanders over time a two way interaction of type of event and time provided the best fitting model with the lowest AIC value. [F (1, 7) = 2.375, P < .021, AIC = 2181.822]. This meant combining all the individual values and these were calculated using the means of the individual animals (see Table 5.8).

For temporary separation (Figure 5.4 A) there was a trend for a reduction in cortisol levels was seen in the group the week prior to and the week following temporary separation, although the levels were very low. There was also a trend for a reduction in cortisol levels for the group for longer than 24hrs from the week prior (Figure 5.4 B), to the day of and in the week following separation. Again these were at low levels when compared to other events. No pattern of change in cortisol levels were seen during reintroduction events (Figure 5.4 C).

Table 5.7

Number of events and number of samples used for each type of separation category.

Role	Type			
	Temporary separation	Separation	Reintroduction	Total
Separated	1	4	7	12
	(6)	(20)	(65)	(91)
Bystanders	1	7	7	15
	(40)	(208)	(197)	(445)
Total incidents	1	7	7	15
(samples)	(46)	(228)	(262)	(536)

Table 5.8

Mean values and number of samples for every individual in each type of separation and time category.

Category	Time	ID						Overall mean	Standard error
		Chr	Mil	Fay	Mar	Ric	Zum		
Temporary separation	Pre	1.584 (3)	2.800 (3)	0.407 (2)	1.835 (4)	0.414 (4)	1.896 (5)	1.489 (21)	0.380
	At	-	-	-	-	-	-	-	-
	Post	0.596 (5)	0.497 (3)	0.502 (5)	1.294 (5)	0.481 (4)	2.157 (3)	0.921 (25)	0.278
Separation	Pre	1.281 (26)	1.746 (7)	2.677 (16)	2.315 (20)	1.459 (26)	2.794 (14)	2.045 (109)	0.261
	At	0.188 (2)	0.839 (1)	1.209 (1)	1.126 (1)	0.365 (3)	1.065 (2)	0.799 (10)	0.174
	Post	1.421 (22)	1.758 (7)	1.953 (9)	3.031 (22)	0.338 (24)	0.935 (17)	1.573 (101)	0.377
Reintroduction	Pre	1.062 (28)	1.310 (12)	0.987 (20)	1.550 (30)	0.489 (21)	2.260 (15)	1.276 (126)	0.244
	At	2.781 (4)	1.540 (4)	0.629 (6)	1.779 (6)	0.365 (3)	2.049 (4)	1.524 (27)	0.368
	Post	1.061 (20)	2.228 (15)	1.093 (21)	1.038 (20)	0.508 (18)	1.541 (17)	1.245 (111)	0.238

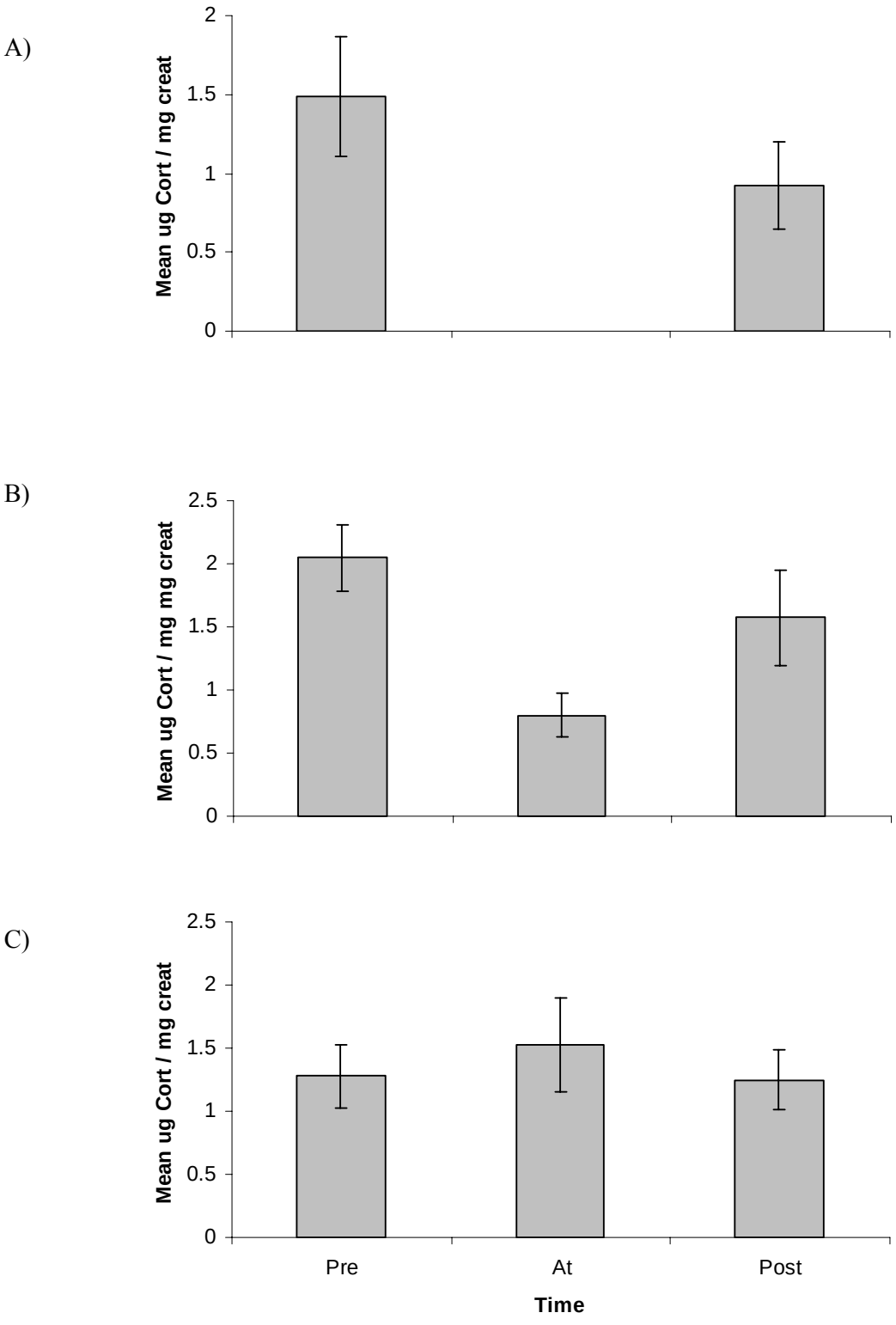

Figure 5.4 Mean values of cortisol over time for all individuals for the three separation events of A) temporary; B) separation and C) reintroduction. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

5.8 Discussion

The results of the present study revealed, for this group of zoo-housed spider monkeys, that different levels of stress response occurred following the three different social contexts of aggression, reproduction and separation. In particular, aggression was more stressful as measured by urinary cortisol than either reproduction or separation.

When I examined aggression only, I found that the patterns of aggression reported in Chapter 4 also occurred in the Chester Zoo group of spider monkeys. Firstly, the predicted pattern of aggression with adult males directing minor aggression towards adult females and severe and lethal aggression towards sub adult males was supported (Asensio, et al., 2008; Aureli & Schaffner, 2008; Campbell, 2003, 2006b; Fedigan & Baxter, 1984; Slater, et al., 2008; Vick, 2008). The majority of the incidents of aggression were by one adult male (Ric). He was responsible for the majority of minor aggressive events, the vast majority of which were directed toward adult females, which is similar to observations found in wild spider monkeys (Fedigan & Baxter, 1984; Slater, et al., 2009). In addition, severe cases of aggression were carried out predominantly by Ric to other adult females, which have not been previously reported. There were, however, incidents of female-female aggression, in particular to the youngest adult female, which also corresponds to patterns of older females targeting younger, newly immigrant females in the wild (Asensio, et al., 2008; Aureli & Schaffner, 2008). Finally, both cases of lethal aggression were by Ric towards juvenile males. Although this was unexpected as Ric was related either as half brother or father to both the males, the pattern of older males targeting and killing younger, potentially related males is also documented in wild populations (Campbell, 2006b; Valero, et al., 2006) and has been reported in other zoo populations of spider monkeys (Chapter 4).

The second prediction that severe cases of aggression would lead to a higher stress response in the targets of aggression was also supported. The LMM was used to demonstrate the best fitting explanation of the data by testing the model that best explains the variance of the dependent variable while controlling for variation due to repeated measures of individuals (Tabachnik & Fidell, 2007). I found a three way interaction of severity, role and time as the best fitting model. For minor aggression

no significant fluctuations in cortisol occurred across time or role. For severe aggression, however, a significant increase occurred in both actors and targets of the aggression on the day of the event. However, the cortisol levels returned to prior levels in the week following severe aggression. As cortisol responses were relatively short lived, providing that the events do not occur on a regular basis, such incidents should be of no concern for the welfare of the individual (Moberg, 2000). I also predicted that for severe aggression, no changes in cortisol would be detected in the bystander which was also supported. These results are similar to those found by Smith and French (1997b) who also looked at role in aggression in marmosets. They found elevated levels of cortisol in both the targets and actors immediately after aggression between siblings, which then returned to baseline within the week. No increases were reported in bystanders.

It was predicted that cases of severe or lethal aggression would be expected by adult males towards sub adult males (Campbell, 2006b; Valero, et al., 2006). It would also be expected that the largest increase in cortisol would be associated with the most severe cases of aggression (Ostner, et al., 2008), with severe and lethal aggression associated with the largest increased levels of cortisol. The largest stress response occurred in bystanders following lethal aggression on the day of the event compared to levels the week prior to the event and, with levels remaining high compared with levels prior to the aggressive event. Although no increase was seen in the actor on the day of the event, an increase was seen in the following week. Some caution may be needed in interpreting these results as this category had the lowest sample size (37 samples) and the high cortisol level for the role of bystander on the day following the event was driven by the high stress response from a mother of a killed infant. Her value on the day following aggression was 67.27 ug cort/mg creat, compared to the mean rate of 1.848 ug cort/ mg creat for other bystander females. Such a response is not surprising as the highest stress responses in female primates are known to occur when they are separated from their infants or if infants die (Boccia, et al., 1995; Engh, et al., 2006; Maestripieri, et al., 2008).

Higher cortisol levels have been associated with levels of aggression in a number of primate studies in captivity (Eberhart, Keverne, & Meller, 1983; T. E. Smith & French, 1997b) and in the wild (G. M. Barrett, et al., 2002; Beehner, et al., 2005; Cavigelli, et al., 2003; Muller & Wrangham, 2004; Ostner, et al., 2008; Sapolsky, 1982). While a number of aggressive incidents were recorded in the spider

monkey group over the six year study period, overall incidents of aggression were relatively low in comparison to other species of primate (Bernstein, et al., 1983; see Chapter 4).

Regarding reproductive events, the first prediction that ovulation or mating events would be associated with an increase in cortisol was not supported, as neither the male nor the females experienced increased cortisol during either of the events. In the case of mating, the findings suggest that although the ability to mate in secrecy was compromised, which is a species-specific characteristic (Campbell & Gibson, 2008), this constraint on normal behaviour was not stressful. Given my own observations and those of others (Schaffner & Aureli, personal communication, March 2009) the Chester Zoo spider monkeys do mate in secrecy. On at least five occasions full copulations have been observed in which the male and female form a consortship away from other group members, remain vigilant during the entire event and manage to successfully copulate without visual knowledge of other group members. It may be the case, in other zoo populations, where secrecy is not possible that a higher stress response is more likely. In addition, the secrecy in mating is believed to be a strategy to avoid mating in the presence of other males (Campbell & Gibson, 2008). The population of spider monkeys housed at Chester Zoo has only one adult male, therefore, it may also be the case that in zoo populations with more than one male, the need to engage in secret copulations is more important and therefore a stress response may be more likely in these populations.

The fact that I did not find an increase in cortisol during ovulation was more surprising. Cortisol levels have been shown to change systematically in primates over a non-conceptive ovarian cycle (Saltzman, et al., 1998). Levels of cortisol increase steadily during the follicular phase, peak during the late follicular, peri-ovulatory, and early luteal phases and decline in the mid- to late luteal phase (Saltzman, Schultz-Darken, Scheffler, Wegner, & Abbott, 1994; T. E. Smith & French, 1997b; Ziegler, et al., 1995). However similar findings were found in marmosets (*C. kuhlii*) by Smith and French (1997b) who found no increases during ovulation which suggests some species variation. The significance and mechanisms of such rises is unknown although it may be affected by the actions of changing levels of other steroid hormones such as progesterone and oestrogen (Beehner, Nguyen, Wango, Alberts, & Altmann, 2006; Saltzman, et al., 1998). That I did not

find such increases may be because my measure of ovulation was mainly behavioural and was therefore not precise.

However, in spider monkeys it is difficult to determine where a female is ovulating because the various behaviours associated with ovulation are not necessarily associated with the peri-ovulatory period (Campbell & Gibson, 2008). It has even been suggested that the concept of oestrus does not apply to spider monkey sexual behaviour as matings have been observed when conception is not possible (Campbell, 2007). For a more accurate assessment of the affect of reproductive cycle on cortisol it would be necessary to focus on the sex hormone profile of females. Further study is also required in this area focusing on using female hormone profiles to determine whether there are more subtle behavioural changes during their ovarian cycle (Campbell & Gibson, 2008).

The final prediction, that the birth of an infant would be associated with increased cortisol was supported, although the increased sensitivity to potential stressors on the mother, postpartum, resulting in heightened GC levels (Behringer, et al., 2009) was not demonstrated. Instead GC levels were at their highest in the week prior to the birth, before falling back on the day of birth and returning to baseline levels for the week following. Pregnancy could be seen as a potential stressor due to increased metabolic costs and this could be related more to the physiological effects of pregnancy (Weingrill, et al., 2004). In humans plasma cortisol increases during pregnancy and then declines following parturition. By late trimester total cortisol levels are approximately three times non pregnant levels, rising to five times in late gestation (Keller-Wood & Wood, 2001). There are also well established links between reproductive status and basal cortisol levels. For example, squirrel monkeys (Vogt, Coe, & Levine, 1981), callitrichids (Saltzman, et al., 1998; T. E. Smith & French, 1997b; Ziegler, et al., 1995) ring-tailed lemurs (*Lemur catta*: (Cavigelli, 1999), chacma and yellow baboons (*Papio hamadryas ursinus, P. cynocephalus*) (Weingrill, et al., 2004), (Beehner, et al., 2006), marmosets (*C. kuhlii*) (T. E. Smith & French, 1997b), (*Callithrix jacchus*) (Tardif, Ziegler, Power, & Layne, 2005) all showing elevated cortisol levels during late gestation. However, the same pattern has not been found in rhesus macaques (Challis, Davies, Benirschke, Hendrickx, & Ryan, 1975). This heightened cortisol at a time of increased metabolic demand might not be surprising considering its metabolic role (Setchell, et al., 2008). An increase in GC levels is also related to the initiation of labour in humans, a mechanism that may

also be found in other primates (R. Smith, Chan, Bowman, Harewood, & Phippard, 1993).

The increase in cortisol levels in the other females at birth was not predicted and was surprising. The timing of the births was checked to ensure that they did not coincide with any other stressful events which may have confounded the results, and no other significant events were found within the sample periods. A possible explanation for the increase may be that the arrival of an additional group member may represent a greater competition for food resources in the future. This post weaning resource competition would apply in particular for male infants in spider monkeys where there is female dispersal (Chapman, et al., 1989). Other possible explanations may be that the increase is related to the effect of novelty which can elicit increases in GC levels (Hennessys, et al., 1995), or an increase in overall activity as proposed when similar results were observed in a group of captive bonobos (Behringer, et al., 2009). A positive correlation between locomotion and levels of cortisol has also been observed in some species (e.g. T. E. Smith, et al., 1998).

Finally, I predicted that separation events would lead to a stress response, following separation, for the target individual. However, this prediction was only partially supported. The best fitting model for the data revealed that there was a two-way interaction of time and the type of separation. However, this was due to a trend for a reduction in cortisol levels following separation, with levels reducing in particular for the separated individual over time. No changes were shown following reintroduction. The reason for the separation needs to be considered. Generally, individuals were separated following an aggressive incident or for veterinary reasons which may mean cortisol levels prior to separation were elevated. Although in previous studies separation has led to a significant stress response (Boccia, et al., 1995; Crockett, et al., 1995). The conditions of the separation in this study meant the individuals were still within visual and tactile contact of the other members of the group which is known to have a social buffering effect (T. E. Smith, et al., 1998). Species differences are known to occur with the effects of social isolation and these may depend on the behavioural and ecological characteristics of the primate (Crockett, et al., 2000). In addition, it may be that because spider monkeys live in a social system characterised by high fission-fusion dynamics and therefore typical to

have individuals travelling on their own for days or weeks at time (Schaffner and Aureli personal communication) such separations are not stressful for this species.

In summary this long term investigation into the effects of various social stressors on a group of zoo-housed spider monkeys found aggressive events to elicit the greatest stress response and that these were affected by severity, time and role. Only for the rare incidents of lethal aggression were there sustained increases in GCs, which may represent distress to the animals concerned. For reproductive events there was no evidence for any stress response associated with ovulation and mating events even though a zoo environment reduces the potential for consortships away from the rest of the group. High GC levels in the week prior to birth were found, but this represents physiological effects of a higher metabolism and has also been found in other primate species during late pregnancy (Setchell, et al., 2008). A high level of GC for other females during birth was unexpected but may be related to the effect of novelty which can elicit increases in GC levels (Hennessys, et al., 1995), or an increase in overall activity as proposed when similar results were observed in a group of captive bonobos (*Pan paniscus*) (Behringer, et al., 2009).

This was a retrospective study and there was no control over the various events studied, therefore, the likelihood of different events having confounding effects on cortisol should be considered. However, the statistical approach used, to examine the various factors take into account the magnitude of the response to the different variables individually in determining the best explanatory model for the data (Tabachnik & Fidell, 2007). However, to be extremely conservative and minimize such confounding factors any lower level events, which overlapped with any major events, were not analysed. However, it is possible that longer term effects of the higher level events may have influenced some of the results.

While enzyme immunoassays have been used previously to examine oestrogen (E1C) and progesterone (PdG) in captive spider monkeys (Campbell, et al., 2001), this is the first time levels of cortisol have been investigated with respect to aggression, reproduction or separation events in spider monkeys. Finally, separations did not evoke a considerable stress response unlike that observed in other species (Hennessys, et al., 1995). In fact it actually led to a significant reduction in GC levels, which may be explained both by the conditions of the separation and the natural fusion-fission characteristic of spider monkey social life. This could have implications for recommendations of management practices for separations or

reintroductions for spider monkeys in zoological parks. Considerable differences have been found between species and sub species in the stress responses of various contexts, which could be caused by the different approaches in responding to stressors (Honess & Marin, 2006a).

CHAPTER 6

INTRODUCTION OF A NEW MALE TO AN ESTABLISHED GROUP OF CAPTIVE SPIDER MONKEYS – A CASE STUDY

6.1 Introduction

For zoological parks to preserve populations of wild animal species over long periods of time it is necessary to maintain their genetic diversity and demographic security (Ballou & Foose, 1996). This means that populations need to be managed with carefully co-ordinated programmes requiring full co-operation across institutions (Hosey, et al., 2009a; Hutchins & Wiese, 1991). The consequences of trying to maintain genetic diversity in what is essentially a series of small and fragmented populations therefore requires the regular movement of individuals across a number of social groups.

Within wild populations dispersal occurs naturally, although there is variation across sex, age and life history stages (Pfeifer, 1996). The main evolutionary factors are to avoid inbreeding, reduce competition over local resources, reduce mate competition and co-operative behaviour among kin, with evolutionary stable patterns of dispersal assumed to result from a balance of these selected forces (Nagy, Heckel, Voigt, & Mayer, 2007). Typically among mammalian species, dispersal is sex-biased in favour of males (Greenwood, 1980), although there are exceptions such as wild dogs, *Lycaon pictus* (Frame & Frame, 1976), chimpanzees, *Pan troglodytes* (Pusey, 1987), traditional agrarian human societies, *Homo sapiens* (Boehm, 1992; Marlowe, 2005) and spider monkeys, *Ateles* spp (Di Fiore & Campbell, 2007; McFarland Symington, 1990). Although some adverse effect of relocating animals to new zoo settings is to be expected, it is essential that the dispersal patterns in natural populations are considered when planning such moves in order to minimise disruption and stress (Pfeifer, 1996).

The movement of animals across populations in captivity can be disruptive and can cause social instability in the existing group (Kleiman, 1980). The formation of new groups and the introduction of new animals into existing groups can be extremely stressful and potentially dangerous to the immigrant and members of the

existing group, particularly in social primates (Brent, et al., 1997; A. S. Clarke, Czekala, & Lindburg, 1995; Reinhardt, Liss, & Stevens, 1995). The separation of individual monkeys which can happen for a period of months prior to and even following the translocation of individuals can also be extremely stressful (Noble, et al., 1976). The consequences of introductions and separations however show significant variation across different species of primates, which may be linked to their social organisation and mating patterns (A. S. Clarke, et al., 1995; Mendoza, et al., 2000) (see Chapter 5).

A number of studies have assessed the impact of group formation, using various indicators of stress including behavioural, physiological and immune responses (M. R. Clarke, et al., 1996; Doyle, et al., 2008; Gust, Gordon, & Hambright, 1993; Line, et al., 1996; Schaffner & Smith, 2005). Studies in the wild have shown significant increases in glucocorticoids (GCs) following the migration of unfamiliar males (Beehner, et al., 2005; Engh, et al., 2006). In captive studies GC levels have also been used alongside behavioural studies in order to assess the impact of group formation. Several studies have been conducted in macaques that examine the impact of changing group composition. Increased GC levels and significant aggressive behaviour occurred in rhesus macaques in response to group changes (*Macaca mulatta*) (M. R. Clarke, et al., 1996; Gust, Gordon, & Hambright, 1993; Westergaard, Izard, Drake, Suomi, & Higley, 1999), and increased GC levels but no serious aggressive behaviour was observed in female pig tailed macaques (*M. nemestrina*) when they were moved from individual cages to form a new group (Gust, et al., 1996). In a study comparing the group formation of two species of male macaques, GC levels decreased over time in cynomolgus macaques (*M. fascicularis*), while lion-tailed macaques (*M. silenus*), who showed more aggression, had GC levels that remained at high levels (A. S. Clarke, et al., 1995). In a study with marmosets (*Callithrix kuhlii*) the formation of multi-male polyandrous groups (using related males) found no changes in GCs *(*Schaffner & French*,* 2004*).*

In an evaluation of introduction procedures in chimpanzees, there was a great deal of variation from one facility to another (Alford, Bloomsmith, Keeling, & Beck, 1995; McDonald, 1994). Chimpanzees share a social organisation, like spider monkeys, which is characterised by a high degree of fission-fusion dynamics (McFarland Symington, 1990). They also demonstrate a fluid social structure with males showing the strongest bonds. In addition, males are not known to transfer

between groups and instead remain in their natal group. In the wild, unfamiliar males are normally met with hostility and inter group aggression can be violent, leading to serious injury and even death (Watts, et al., 2006; M. L. Wilson, Wallauer, & Pusey, 2004; M. L. Wilson & Wrangham, 2003). With their size, strength and natural aggressive tendencies towards unfamiliar males, introductions in chimpanzee groups in captivity can often be difficult. The dangers are confounded by typical captive environments which are confined, providing fewer opportunities for individuals to avoid conflict or escape aggression. Atypical species groupings and the high frequency of movement between groups of chimpanzees are also probably related to an increased instability and aggression in the captive environment (Brent, et al., 1997).

6.2 Factors influencing introductions

Various strategies have been adopted for the introduction of primates into groups or the formation of new groups. For rhesus macaques simultaneous introductions resulted in high rates of serious aggression that resulted in high mortality rate (Bernstein, et al., 1983). Familiarisation of potential group members prior to introductions has been tried with four species of macaques with mixed results (Reinhardt, et al., 1995). The timing of familiarisation is also important so that the animals can establish rank relationships, but not too long as to exacerbate initial fear response if resolution is not achieved (Brent, et al., 1997; Reinhardt, et al., 1995). Repeated attempts to introduce rhesus macaques sometimes led to increasing tension between animals (Bernstein, 1991). However, if introductions were carried out gradually with a small number of animals at a time agonistic behaviours were greatly reduced in this species with higher rates of grooming and sexual interactions (Westergaard, et al., 1999). The size of the group can also be a factor, with the response from a group of black tufted-ear marmosets (*Callithrix kuhli*) to the introduction of a new female varying with group size, with a more aggressive response found in larger groups (T. E. Smith & French, 1997b).

The design of the enclosure during group formation can also be important (Brent, et al., 1997; Westergaard, et al., 1999). Aggression and injuries in chimpanzees were lower in facilities where the design allowed for dyadic pairing and gradual introductions, with initial visual contact, followed by limited tactile contact

before full contact (Alford, et al., 1995; McDonald, 1994). In rhesus macaques wounding rates were higher if there were no visual or social barriers present during introductions (Westergaard, et al., 1999).

The sex of the individual seems to be an influential factor. Introductions of male chimpanzees were much more likely to be unsuccessful than those involving females (Brent, et al., 1997). This fits in with the social organisation of chimpanzees in which males respond with affiliative behaviour toward newly immigrant females. Similar patterns were also found in other non human primates (Bernstein, 1991; Crockett, et al., 1994). Prior familiarity of the individuals may significantly reduce the impact of forming new groups (Schaffner & French, 2004; Schaffner & Smith, 2005). Other factors include previous social housing and age at introduction (Reinhardt, et al., 1995).

6.3 Spider monkeys

The dispersal pattern for spider monkeys is one of the few exceptions in mammals in which female emigration is the rule and males remain in their natal group all of their lives (Aureli & Schaffner, 2008; Greenwood, 1980; see Chapter 2). However, in zoo populations, to maintain genetic diversity, it is common practice for the males to be transferred across facilities with females remaining in their natal group. The consequences of this unnatural dispersal pattern in spider monkeys across zoological parks has been investigated (see Chapter 4), however, its effect on the behaviour and stress response is unknown and has not previously been assessed.

Based on the social ecology of spider monkeys, particularly regarding female dispersal, and previous studies involving introductions of other primate species in captivity in which abrupt introductions have lead to serious and occasional lethal aggression (Crockett, et al., 1994; Reinhardt, et al., 1995) I made two predictions. The first was that the introduction of the new male into an established group of female spider monkeys and their offspring would initiate a stress response in the residents and the new male. I further predicted that behavioural indices of stress, such as self-directed behaviour would be more evident immediately after the male was introduced. In this study, the effect of the introduction process and following

period was assessed for eight weeks using behavioural observations and urinary cortisol to measure activity of the HPA axis.

6.4 Aim

The aim of this investigation was to examine the behavioural and adrenocortical responses of all adult and sub adult group members to the introduction of a replacement adult male into an established breeding spider monkey group within a zoo environment. Behavioural observations and the collection of urine commenced two weeks prior to the arrival of the new male and continued for six weeks following his arrival to assess changes in behaviour, proximity and cortisol levels of individuals within the group throughout the introduction period. To reduce the impact a gradual introduction technique was used to allow for a period of familiarisation prior to the full introduction. The new male was housed in a separated area of the enclosure that allowed the opportunity for full visual and tactile contact with the rest of the group (see Chapter 2, Figure 2.4 and 2.5).

6.5 Methods

6.5.1 Subjects

The group size over the study period was made up of 11 individuals, briefly dropping to 10 between the relocation of the original male and the introduction of the new male. The group was made up of one adult male, five adult females, four juveniles and one infant (see Table 6.1).

6.5.2 Procedure

6.5.2.1 Urine collection

Urine collection occurred throughout the eight week study period and followed the protocol outlined in Chapter 2.

Table 6.1

Monkeys that served as subjects in the experiment.

ID	Date of birth	Sex	Change of group membership over study period
Ric	10.08.97	Male	Relocated – 07.03.08
Mar*	1970*	Female	Resident
Chr	30.09.88	Female	Resident
Poy	03.09.89	Male	Introduced – 11.03.08
Zum	06.12.93	Female	Resident
Fay	22.02.94	Female	Resident
Naj	16.02.02	Female	Resident
All	28.09.03	Female	Resident
Pop	01.12.04	Male	Resident
Syd	05.08.06	Male	Resident
Win	17.08.07	Female	Resident
Fel	03.01.08	Male	Resident

*Birth date unknown, wild caught, arrived in Chester Zoo in 1982 as adult individual likely > 10 years of age at that time

6.5.2.2 Observations

The study period was divided into six separate time categories to reflect the ongoing events. Pre-introduction represented the control period before the resident male was moved (27.02.08 – 06.03.08); 'no male' represented the time of no adult male in the group i.e. between when the resident male was relocated and before the arrival of the new male, Poy (07.03.08 – 10.03.08); 'Poy back' was the time following the arrival of the new male when he was housed separately at the back of the enclosure, but in full visual and potential tactile contact with other members of the group (11.03.08 – 13.03.08); 'introduction' was the period when the new male was introduced with the rest of the group, and included a temporary separation following a fight (13.03.08 – 17.03.08); 'inside' was the time when the group was housed together, but Poy was not permitted to go to the outside enclosure, therefore there were brief periods for a few hours during several of the observation days when the male was separated into the rear part of the enclosure to allow the rest of the

group access to the outside enclosure (17.03.08 – 06.04.08); and finally 'outside', which represented full 24hr integration of the male with the group with full access to indoor and outdoor enclosures (07.04.08 – 27.04.08). Each focal animal was observed for a 10 minute focal with continuous recording used in which its behaviour and proximity to other members of the group was recorded (see Table 6.2 and 6.3) (Martin & Bateson, 2007). The data were entered onto a check sheet (see Appendix D) to be later entered for data analyses. I carried out the observations from one of three time periods, 08:00 – 10:59, 11:00 – 13:59 and 14:00 –17:00. This was done to take account of any differences in behaviour patterns of the spider monkeys during the day that could have been affected by external factors, such as keeper routine and visitor numbers. Each individual was selected at random, but only observed once per time period. The number of focal observations per time period was not fixed. Of the 61 days of the study observations occurred on 49 days. The ethogram follows Schaffner and Aureli (2005) developed for this same group of spider monkeys. Due to the infrequency of agonistic behaviours and embraces they were not included on the ethogram, although were recorded as they occurred.

6.5.3 Analyses

Over the study period, urinary cortisol levels and behaviour were investigated using linear mixed models (LMM's). LMM's were applied following the procedures laid out in Chapter 5. For this investigation, Maximum likelihood (ML) method was used. The state behaviours and proximity data were converted to proportions and transformed for statistical analyses using ARCSINE square root transformations (Martin & Bateson, 2007). Mean proportions were calculated by taking the proportion for each behaviour from each individual for each time period and then calculating the overall mean proportion from each phase of the study.

Scratches were analysed as mean number per time category. These values were entered as a continuous dependent variable, time as the fixed variable and identity as the random variable in the models. The cortisol values were also entered as a continuous variable. The resident individuals were analysed separately from the data obtained from the resident and new male. When a main effect of time was detected, post hoc comparisons were carried out using Bonferroni's test. An alpha level of 0.05 was adopted for all inferential statistical analyses.

Table 6.2

Ethogram.

Behaviour	Definition
Individual/self-directed behaviour	
Feeding	Monkey masticates food while food is in hand or mouth or systematically searches for food by manipulating substrates with hand or by peering directly toward floor of enclosure with head moving slightly back and forth
Resting	Monkey is stationary on substrate, either lying or sitting, included times when eyes were closed
Locomotion	Monkey moves about the enclosure by walking, running or brachiating
Auto grooming	Monkey manipulates its own fur with hands or mouth and was considered the same auto grooming bout if the individual resumed auto grooming within 5 seconds even if another part of the body was auto groomed
No contact	Resting more than an arms reach from another
Self scratching	Repeated scraping of fingers on individual's own fur or body if individual resumed scratching within 5 seconds even if another part of the body it was considered the same scratch
Social behaviours	
Groom other	One monkey manipulated the fur of another individual with its hands or mouth and was considered the same groom bout if the individual resumed grooming the other within 5 seconds even if another part of the body
In proximity	Resting within an arm's reach from another
In contact	Resting in bodily contact with another (including infants)

Table 6.3

Number of observations during each phase of the study.

ID	Pre introduction	No male	Po back	Introduction	Inside	Outside
Ric	7	N/A	N/A	N/A	N/A	N/A
Mar	6	3	5	8	12	15
Chr	8	3	5	7	10	14
Po	N/A	N/A	5	7	13	16
Zum	8	3	5	7	14	14
Fay	6	3	5	7	14	16
Naj	5	3	5	6	15	14

The data for males were presented as descriptive statistics (mean ± standard deviation). Differences in the behaviour of the new male over time or between the new male and the resident male were determined when the mean rate ± standard deviation did not overlap between any two sets of scores.

The first set of analyses examined changes within individuals in the three proximity categories of 'no contact', 'proximity' (within arms reach) and 'in contact' over the six time categories. The second set of analyses examined changes over time in the six different behaviours of 'feeding', 'resting', 'locomotion', 'auto grooming', 'groom other' and 'self scratching'. The third and final set of analyses examined changes in levels of cortisol over the study period. Post hoc tests used pairwise comparisons and adopted the Bonferroni's correction.

6.6 Results

6.6.1 Social behaviours

Analyses demonstrated that the proportion of time individual residents spent in 'no contact' with another individual changed significantly over the six time categories [F (1, 5) = 5.666, p < .001, AIC = 229.377, see Figure 6.1A]. Post hoc analysis revealed a significant decrease from 'Pre-introduction to 'Poy back' (p <

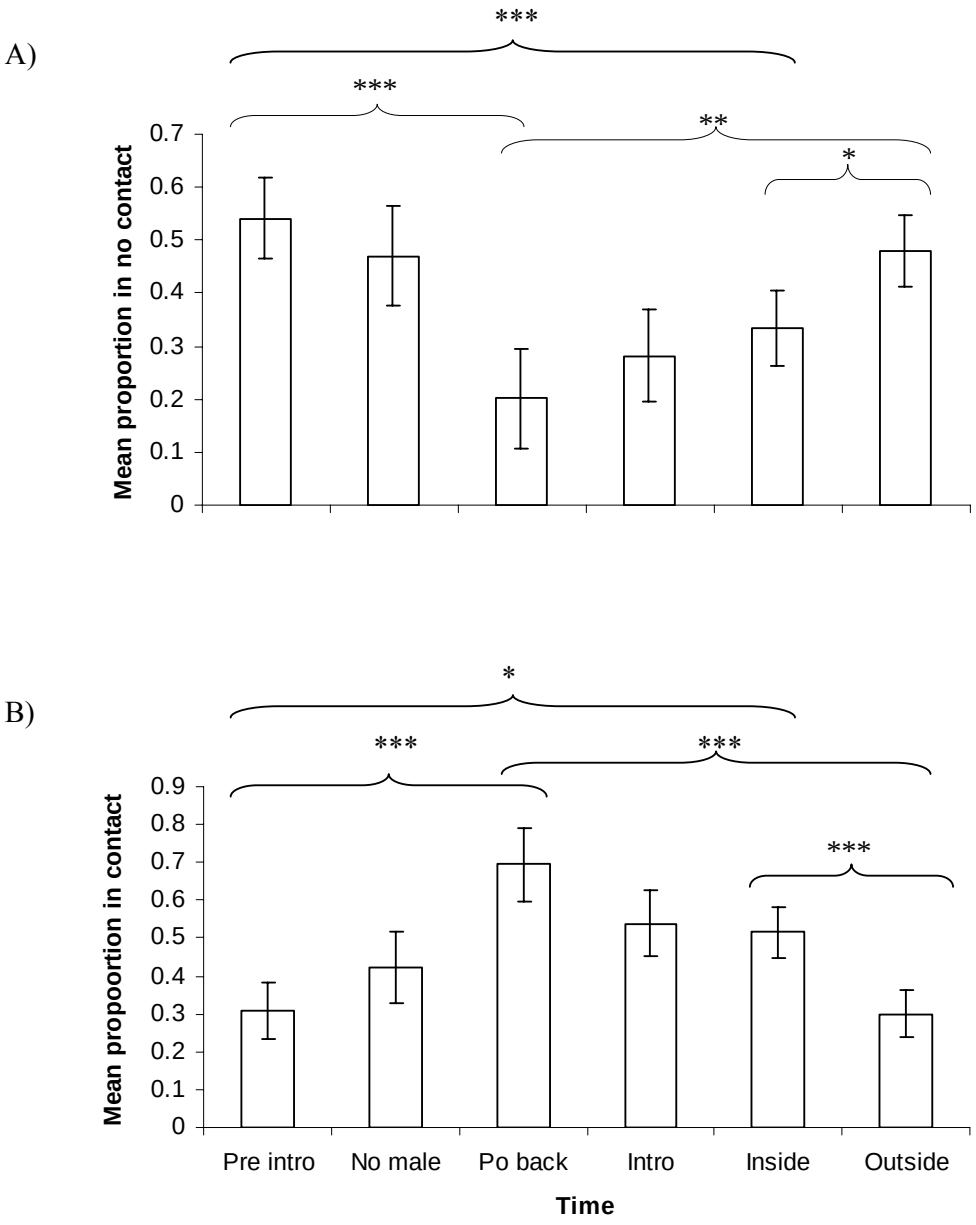

Figure 6.1 Mean proportion of observation time for female resident spider monkeys A) in no contact and B) in contact. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

.005), and 'Pre-introduction and inside' (p <0 .005). There was also an increase from 'Poy back' to 'outside' ($p < .001$) and 'inside' and 'outside' ($p > .05$). Not surprisingly, the change in the proportion of time females were not in contact appeared to correspond to the pattern of proportion of time they spent in contact with each other, which also changed over time [F (1, 5) = 7.277, $P < .001$, AIC = 274.757, see Figure 6.1B]. Post hoc analysis revealed a significant increase between 'pre-introduction and 'Poy back' ($p < 0.05$), and 'pre-introduction' and 'inside' ($p < 0.05$); and a significant decrease between 'Poy back' and 'outside' and 'inside' and 'outside' (both comparison, $p < .01$). There was no significant difference detected over time for proximity [F (1, 5) = 1.80, P = 0.114, AIC = 99.179, Table 6.4].

Table 6.4

Mean proportions of observation time for female resident behaviours that did not change significantly over the study period.

Behaviour	Time category (mean values ± SEM)					
	Pre introduction	No male	Poy back	Introduction	Inside	Outside
Social behaviours						
In proximity	0.147 ± 0.04	0.107 ± 0.05	0.104 ± 0.05	0.179 ± 0.05	0.152 ± 0.03	0.220 ± 0.03
Individual behaviour						
Feeding	0.183 ± 0.05	0.176 ± 0.06	0.082 ± 0.06	0.125 ± 0.05	0.142 ± 0.04	0.162 ± 0.03
Resting	0.663 ± 0.05	0.63 ± 0.07	0.820 ± 0.07	0.698 ± 0.07	0.710 ± 0.05	0.691 ± 0.04
Locomotion	0.105 ± 0.02	0.113 ± 0.03	0.066 ± 0.03	0.113 ± 0.02	0.072 ± 0.02	0.106 ± 0.02
Auto grooming	0.013 ± 0.01	0.006 ± 0.03	0.042 ± 0.02	0.041 ± 0.02	0.019 ± 0.01	0.033 ± 0.01

For the males there was a difference in the proportion of time spent in 'no contact' with another individual for the two males (see Table 6.5). The newly introduced male spent a greater proportion of his time in 'no contact' than did the previous resident male (Table 6.5). There was also a change in the proportion of time

the former resident male spent 'in contact' with other group members compared to the new male (Table 6.5) The new male spent almost no time in contact with residents across the duration of the study period, however as time progressed, he spent a greater proportion of his time in proximity to other residents, particularly during the time period when all individuals were confined to the indoor part of the enclosure (Table 6.5).

Table 6.5

Changes in the mean proportions of observation time for new male's behaviour over the study period and for Ric in the Pre introduction phase.

Behaviour	Time category (mean values ± SEM)				
	Pre introduction	Poy back	Introduction	Inside	Outside
Social behaviours					
Groom other	0 ± 0.01	0 ± 0.01	0 ± 0.01	0.013 ± 0.01	0.004 ± 0.01
In proximity	0.074 ± 0.07	0 ± 010	0.265 ± 0.09	0.180 ± 0.05	0.068 ± 0.04
In contact	0.237 ± 0.04	0 ± 0.06	0 ± 0.05	0.013 ± 0.03	0.015 ± 0.03
No contact	0.689 ± 0.08	1 ± 0.12	0.735 ± 0.10	0.807 ± 0.06	0.916 ± 0.05
Self-directed behaviour					
Auto grooming	0.118 ± 0.02	0 ± 0.03	0.013 ± 0.03	0.001 ± 0.02	0 ± 0.01
Self scratching	0.469 ± 0.08	2 ± 0.14	1.222 ± 0.17	0.26 ± 0.06	0.182 ± 0.06
Individual behaviour					
Feeding	0.015 ± 0.05	0.214 ± 0.08	0.153 ± 0.07	0.085 ± 0.04	0.100 ± 0.04
Resting	0.750 ± 0.09	0.700 ± 0.13	0.717 ± 0.11	0.764 ± 0.07	0.591 ± 0.06
Locomotion	0.116 ± 0.06	0.086 ± 0.10	0.117 ± 0.08	0.138 ± 0.05	0.306 ± 0.04
No contact	0.689 ± 0.08	1 ± 0.12	0.735 ± 0.10	0.807 ± 0.06	0.916 ± 0.05

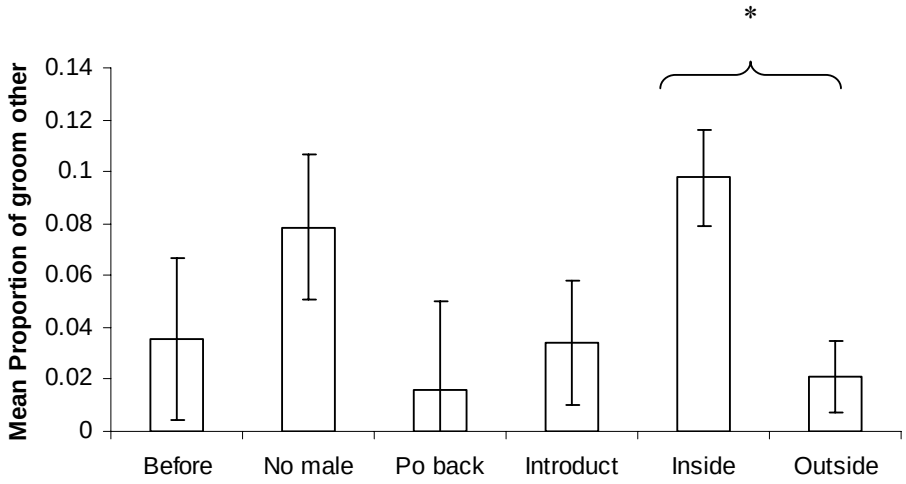

Figure 6.2 Mean proportion of observation time in female resident monkeys for groom other. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

For resident females, mean values of behaviour revealed that 'groom other' showed a significant change over time [F (1, 5) = 2.422, P < .0001, AIC = -49.718, see Figure 6.2], with a significant decrease between the time category 'inside' and 'outside' (p < .05). For the males there was more 'groom other' behaviour by Poy during the inside phase, which then reduced in the outside phase (see Table 6.4).

6.6.2 Self directed behaviour

For the resident females, 'self scratching' showed a significant change over time [F (1, 5) = 5.936, p < .001, AIC = 667.353, see Figure 6.3] with significant increases from 'before' to 'Poy back' (p <.005) and 'no male' to 'Poy back' (p <.005), and a decrease from 'Poy back' to 'inside' (p <.005) and 'outside' (p <.005). Locomotion approached a significant effect over time, but none of the post hoc comparisons yielded significant differences [F (1, 5) = 2.099, p = 0.07, AIC = -130.01, see Table 6.4].

For the males, Poy had a higher level of 'self scratching' following his arrival as compared to Ric, during the 'Poy back' and introduction phases, which then reduced over the study period to levels similar to Ric's in the pre-introduction phase

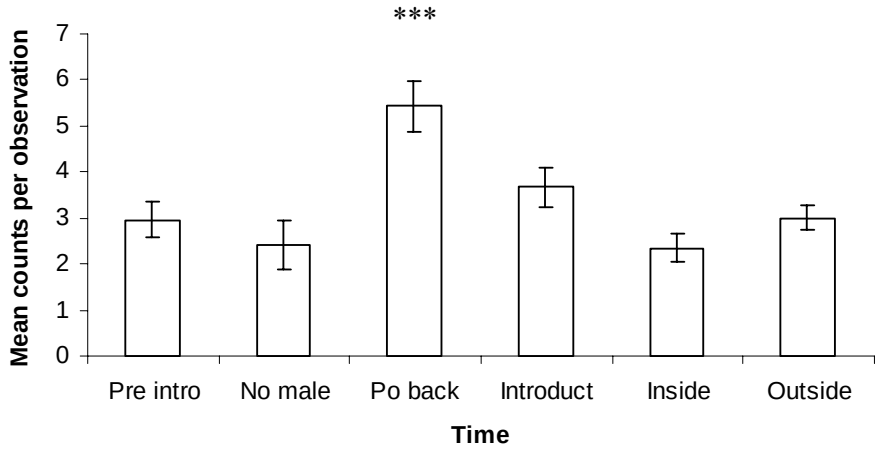

Figure 6.3 Mean proportion of observation time in female resident monkeys for self-scratching. Vertical lines depict standard errors of the means. See text for explanation of significant effects.

(Table 6.5). Although frequencies were very small, lower levels of 'auto grooming' were recorded for Poy than Ric.

6.6.3 Individual behaviour

For female residents feeding [F (1, 5) = 0.311, p = 0.91, AIC = 112.983, see Table 6.4], resting [F (1, 5) = 5.936, p = 0.24, AIC = 175.007, see Table 6.4], and auto grooming [F (1, 5) = 5.936, p = 0.449, AIC = -124.180, see Table 6.4] did not vary over the different phases of the study, although locomotion did approach significance [F (1, 5) = 2.098, p = 0.067, AIC = -130.008, see Table 6.4].

For the males, the mean values of behaviour differed between the values of the previous male (Ric), and new male (Poy) in the amount if time spent 'feeding' with Poy spending considerably longer period of time feeding or foraging for food; and an increase in 'locomotion' when Poy had access to outside (see Table 6.5).

6.6.4 Cortisol

Cortisol values for resident females revealed significant fluctuations over time [F (1, 5) = 1.928, p =.009, AIC = 388.858, see Figure 6.4], although post hoc comparisons did not reveal significant differences among the different phases of the study. Although the values for the males showed a higher level of cortisol in Poy compared to Ric the difference was not meaningful. However, Poy had a higher cortisol level when he was in the back of the enclosure compared to when he was fully introduced (see Table 6.6).

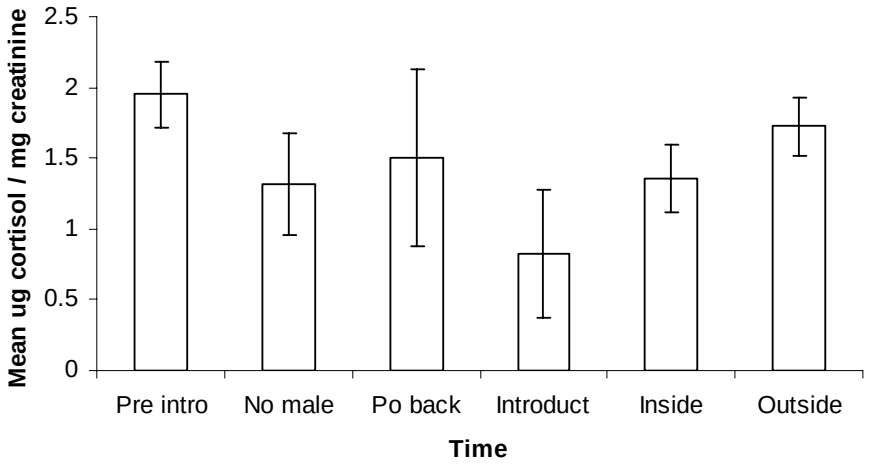

Figure 6.4 Mean urinary cortisol levels o over time for female residents. Vertical lines depict standard errors of the means.

6.7 Discussion

This study represents a multidisciplinary approach to assessing the stress response in a group of spider monkeys following the changing of a new male in an established group. Over the eight week period there were some changes in behaviour. Of particular interest is the initial increase and then decrease of the scratching behaviour among the resident females and the newly introduced male.

Firstly, the introduction of a new adult male spider monkey led to a variety of changes in the proximity dynamics for residents. The arrival of the new male was

Table 6.6

Mean values of urinary cortisol for the previous male (Ric) and new male (Poy) over the study period.

Behaviour	Time category (mean values)				
	Pre introduction	Po back	Introduction	Inside*	Outside
Ric	1.907 ± 0.40	NA	NA	N.A.	NA
Poy	NA	2.44 ± 1.07	0.795 ± 1.07	N.A	1.426 ± 0.404

*no samples were available from Poy as it was not possible to collect them from him

associated initially with a significant increase in the proportion of time the female residents spent 'in contact' with other group members as compared to the pre-introduction period with the original male. Over the following phases of the study this proportion gradually decreased, finally returning back to control levels in the last time period. An associated inverse pattern was also found for the proportion of time spent by the resident females in 'no contact' with a significant decrease from the control period to the period of the arrival of the new male. These proportions then increased over time with the final period again illustrating levels similar to the pre-introduction period. There was also a trend for an increase 'in proximity', representing the number of residents within arms reach, following the introduction of the new male. This increase in contact is consistent with other studies that show that the presence of significant social partners can have beneficial effects during the exposure to a physical or psychological stressor (T. E. Smith, et al., 1998; see Chapter 5).

For the new male there was a lower proportion of time spent 'in contact' that he spent with other members of the group compared to the previous resident male, dropping to almost zero at the time when the new male was introduced. By the end of the study there was a slight increase, although this was still very small compared to the rate of contact that the resident male had with other members of the group. There was also a corresponding change in time spent in no contact. Finally, there was a pattern of a reduction of time 'in proximity' over time. Contact and proximity towards the new male was almost exclusively by the sub adults and juveniles in the

group, and this decrease may be explained by an initial interest in the new male waning. It is possible that these changes in proximity may have been influenced by the restrictions to the use of the enclosure, for example during the 'introduction' and 'inside' phase the group had only limited access to the outdoor enclosure. However, during the time of year when the study took place, the spider monkeys normally spend the majority of their time in the inside enclosure.

There were also significant changes in some self directed behaviours over the study period. There was a pattern of change for 'self scratching' behaviour in resident females, with an increase following the arrival of the new male as compared to the control, before a decrease back to control levels by the end of the study. The new male showed a gradual decrease in scratching rates over time. The new male also spent a considerably smaller proportion of time 'auto grooming' as compared to the previous male although this may be due to individual variation. Scratching and other self-directed behaviours have been used as an indicator of anxiety in other primate studies (Carder & Semple, 2008; Maestripieri, 2000; Maestripieri, et al., 1992), and its pattern in this study seems to demonstrate such a link.

For the resident females there were fluctuations in groom other over time. There was a significantly high rate of grooming when they were restricted to the inside enclosure only, although there was no consistent pattern over time. This is not unexpected as grooming has been identified as an important tool in primate social relationships (Dunbar, 1988), in reducing tension and in mediating the adverse effects of conflict and aggression (Aureli, Preston, & de Waal, 1999; L. Barrett, et al., 2002). It was interesting that groom other was not significantly higher when the new male was in the back or first introduced to the females. However, given females were in close social contact with each other, this may have served the same reassuring function. In addition, the females may have been more focussed on monitoring the activity of the new male, which would have made grooming others an incompatible task. However, I did not score gaze direction or scanning in this study. Furthermore, groom other may not have the same importance in regulating social relationships in spider monkeys as has been identified in other primate species (Schaffner & Aureli, 2005). The increase in grooming in females might have also reflected changes in the dynamics of female relationship regulation due to confinement in a smaller space (Caws & Aureli, 2003).

Regarding locomotion, there were no significant changes over time for either resident females or males. An increase in locomotion has been proposed as a potential indicator of stress in response to a variety of stressors (T. E. Smith, et al., 1998). For example, increases in locomotion have been found to be positively correlated with increases in urinary cortisol in marmosets during movement to a novel cage (T. E. Smith, et al., 1998). This study does not seem to illustrate such a link, but restrictions on movement due to being restricted to the indoor part of the enclosure may have had an impact. No changes were found over the study period for the proportion of time spent resting or feeding in either resident females or males.

Cortisol levels in the resident females were significantly affected by time but the expected increase following the arrival and the introduction of the new male was not apparent. Although post hoc tests did not reveal any significant changes across time there was actually a decrease in mean values of cortisol following the departure of the old male, and were actually at their lowest at the period of introduction, before rising again following full integration. The overall stress response was not as dramatic as seen in previous studies for other stressors and was comparable to events considered less severe, such as incidents of minor aggression, ovulation and temporary separation (Chapter 5) and this may be due to a number of reasons.

That cortisol levels fell following the departure of the previous male suggests that he may have been a source of stress for some of the resident females. This may not be surprising following the catalogue of aggressive incidents for which he was responsible (see Chapter 5). That the mean levels of cortisol did not increase greatly following the arrival of the new male suggests he was not seen as a threat, even though most of the adult females still had offspring in the group. That no aggressive behaviour was ever observed by him towards the other members of the group, and that throughout the study period only one aggressive incident occurred also supports this. This is in contrast to that recorded for introductions and group formations in other captive primates where aggression and injuries are frequent (Brent, et al., 1997; M. R. Clarke, et al., 1996; Gust, Gordon, & Hambright, 1993; Westergaard, et al., 1999).

The aggressive event happened on the morning of the third day after the introduction with the group. The new male had approached a sub adult female when she was feeding and she responded with a small fear vocalisation. This small cry led to all of the adult females simultaneously launching a prolonged attack against the

new male lasting for around 30 seconds. The fight resulted in minor cuts to two adult females (Mar and Chr) and to the separation of the male for a further 24 hours. Apart from the hours immediately following the fight the new male showed no obvious signs of anxiety throughout the study period and tended to keep away from other members of the group. The highest cortisol values were recorded in the new male immediately following his arrival, although the levels were not excessively high and later decreased. The low numbers of samples obtained from the new male over this study period was due to his reluctance to enter the tunnel for sample collection. This makes it difficult to know the extent of his physiological response to relocation.

Although there were no significant changes in cortisol in the resident females the significant changes in behaviour suggest that the introduction of the new male did evoke a stress response. The behavioural response is normally the first reaction by an animal with an attempt to avoid the stressor by simply removing itself from the threat (Sapolsky, 2000; see Chapter 1). This along with the social buffering effect of significant partners appeared to have reduced the need for an endocrinological response and demonstrates the importance of social partners when exposed to a stressor (T. E. Smith, et al., 1998). It would therefore have been expected that the new male without the benefit of an established social partner would have evoked an increased stress response. Unfortunately the lack of samples from the male in this study means that his endocrinological stress response could not be measured. Over the study period the male spent very little time in contact with the residents although there was a gradual increase in proximity. A follow up study would have been beneficial in establishing the overall time period for full acceptance with the resident group.

The likelihood of a successful introduction of an individual into a social group is dependent on a number of factors. Firstly, it is important that the species natural behavioural characteristics and social organisation are considered (Pfeifer, 1996). In this case the introduction of a male into a group of females is not what would be expected in the wild, with immigration normally carried out by the maturing females (Aureli & Schaffner, 2008; Fedigan & Baxter, 1984), although recent evidence from the field suggests some male immigration may occasionally occur when there are sufficient vacancies for breeding males in a given community of spider monkeys (Aureli & Schaffner personal communication). The fission fusion

social organisation of spider monkeys may have also reduced the impact of the separation of the new male as they are more adapted to deal with such separations.

Individual characteristics and temperament may also be an important factor with certain individuals being more suited to successful introduction (Wemelsfelder, 2007). The new male appeared much less aggressive than the previous male, and this can be influenced by a variety of factors including age, sex, previous experience and previous housing. Based on the spider monkeys' social organisation the introduction of another female into the group would not be expected to elicit a significant negative response in males, although resident females may aggressively target new residents initially (Asensio, et al., 2008; Slater, et al., 2009). If the current introduction had occurred while the previous male was still resident then integration into the group would have been much more difficult, with similar difficulties as reported in chimpanzees in captivity (Alford, et al., 1995; McDonald, 1994).

The relocation of animals between zoological parks is essential to ensure the genetic diversity of the populations of endangered animals is maximized. However, the consequences of such moves can be stressful for the relocated animals and the resident group members in which they are to be introduced (Kleiman, 1980). The monitoring of GC levels has been identified as a practical tool to identify the stress response following the relocation of animals and their subsequent introduction (Schaffner & Smith, 2005). A few previous studies have examined the stress response of relocation of animals between zoological parks (Goymann, Mostl, Van't Hof, East, & Hofer, 1999; Laws, et al., 2007), but they are generally short term studies looking primarily at the effect of transportation rather than the effect of an introduction on the individuals. These current results show that this introduction of an adult male into an established breeding group of adult females and their offspring did result in an impact on the adult females HPA axis, associated with a stress response, although not in the manner predicted. There were also associated changes in some aspects of their behaviour. However, these changes were not comparable to previous stressful events such as aggressive incidents that had occurred at other times during the overall study (see Chapter 5), and so not enough to suggest that this particular introduction resulted in sustained levels of stress that would be deemed bad for their overall welfare (Moberg, 2000). In fact such controlled events may even be beneficial to the monkeys by providing a social stimulus and interest (Chamove & Moodie, 1990).

The technique of a gradual introduction although still eliciting an increase in GC levels for the resident females along with some associated behavioural changes seemed to follow previous studies in captive primates (Brent, et al., 1997; Reinhardt, et al., 1995) and reduce the overall impact of the introduction. By having the facility to separate individuals from a group but to remain in full visual and tactile contact helps facilitate what can be potentially stressful events such as introductions and should be recommended as a management technique for future introductions in spider monkey groups.

CHAPTER 7

DISCUSSION

7.1 Thesis aims

Colombian black faced spider monkeys (*Ateles geoffroyi rufiventris*) face a real threat from extinction and their conservation status has been recently changed to critically endangered (Cuarón, Shedden, et al., 2008). The management of the captive population of this sub species is therefore crucial in order to maintain a long-term viable population as well as for their educational and research value (WAZA, 2005). In order to be able to provide an appropriate environment, which enhances the physical and emotional wellbeing of the individuals, it is essential that a better understanding of their needs is gained (Robinson, 1998). Despite their conservation value, little attention has been paid to this sub-species in captivity with only a handful of studies published (e.g. Campbell, et al., 2001; Eisenberg, 1976; Eisenberg & Kuehn, 1966; L. L. Klein & Klein, 1971; Konstant, et al., 1985; Schaffner & Aureli, 2005). Therefore, the aim of my thesis was to investigate the social and environmental factors influencing the wellbeing of spider monkeys kept in zoological parks. This series of studies adopted primarily a physiological approach that entailed measuring cortisol in urine samples collected over a seven year period to assess the impact of a variety of social and environmental conditions. These studies were supported by behavioural observations as well as a questionnaire study to collect information from other zoological parks around the world that maintain groups of spider monkeys. My findings are important for the long term welfare and have important implications for the management of this primate taxon in zoological parks.

7.2 The use of urinary cortisol to assess the stress response

During the first stage of the study I developed a mechanism to use urinary cortisol as a means of measuring the activity of the hypothalamic-pituitary-adrenal

(HPA) axis to assess physiological stress responses in spider monkeys. I achieved this through the validation of an enzyme immunoassay to quantify levels of cortisol excreted in the urine of spider monkeys (Chapter 2). Since I was able to show that levels of cortisol excreted in the urine accurately reflect levels of circulating cortisol, this then enabled me to assess a variety of potentially stressful events that occurred in the Chester Zoo group over the seven year study period.

7.3 Environmental stressors

The next stage of the study looked at one of the main environmental characteristics that defines a zoo environment, its visitors (Hosey, 2005). While the consensus from behavioural studies is that zoo visitors have a negative impact on primates, which may be detrimental to their welfare (Hosey, 2005), the interpretation of behavioural indices of stress is complex (Rushen, 2000). The impact of a chronic presence of large numbers of unfamiliar humans on the welfare of zoo primates through physiological measures was previously unknown. The effect of visitors on zoo primates, with their closer taxonomic links to humans may be more pronounced than that for other taxa (Hosey, et al., 2009b). Previously, only one study had examined the potential effect of visitors on the physiological aspect of stress regarding the levels of GCs and therefore the activity of the HPA axis (Kalthoff, et al., 2001). This study used salivary cortisol in a number of mammal species and no visitor effect was found. The results from my research revealed a significant effect of visitor numbers on urinary cortisol in spider monkeys, with cortisol levels rising with higher visitor numbers. However, whether this increase in cortisol represented a negative impact on their welfare is difficult to assess. In comparison to the known stressful events such as lethal aggression (values range from 0.453 – 67.27 ug cortisol / mg creatinine) the cortisol levels, which ranged from 1.73 -13.73 ug cortisol / mg creatinine, under high visitor conditions are comparatively low. However, the effect of a prolonged exposure to high visitor numbers has the potential to have an impact on their welfare as it could leave the animal in a prepathological state, which leaves it more susceptible to other stressors (Moberg, 2000). That a stress response was found in the spider monkeys in an enclosure that is relatively large and complex, and which allows the individuals a degree of choice and control

to move to areas away from zoo visitors, is indicative of just how potentially stressful large numbers of zoo visitors may be. It raises potential concerns about how spider monkeys housed in smaller traditional enclosures, which do not include such escape opportunities, may cope. This physiological effect highlights the need to consider the location of visitor viewing areas during the design of new zoo enclosures, and in the improvement of existing enclosures, which can have significant welfare implications.

7.4 Social stressors

Social stressors are particularly effective in producing chronic changes in the HPA axis in non human primates, although this is influenced by the species' social organisation and an individual's position within it (Engh, et al., 2006; T. E. Smith & French, 1997a; Ziegler, et al., 1995). Previous studies of primate aggression have primarily been carried out on group living species characterised with dominance hierarchies. Spider monkey social organisation is unusual in that they live in communities that have a high fission-fusion dynamic which has probably evolved as a means of dealing with competition for resources and social relationships (Aureli & Schaffner, 2008). This makes them an interesting test case for assessing various social factors that may have different impacts on their HPA responses. No previous studies carried out on *Ateles* have examined the relationship between aggression and their GC response. In a zoo environment where space is severely limited opportunities for fission can be extremely limited, and the impact of this restriction on the social relationships of spider monkeys was investigated. I carried out three different studies which examined the effect of various social dynamics on cortisol and behaviour in spider monkeys.

The first study followed incidents of aggression at Chester Zoo and assessed by means of a questionnaire (Chapter 4) the severity, context and direction of any aggression in groups of zoo-housed spider monkeys from 24 different zoological parks world wide. In the second study, I assessed how the activity of the HPA axis in individual spider monkeys fluctuated in response to aggression, reproductive events and social separation via urinary cortisol. Finally, I examined the impact of introducing a new male into the group of spider monkeys at Chester Zoo, and measured both behavioural and hormonal activity.

Several aspects of these studies are important for understanding how social dynamics impact on the welfare of spider monkeys. The most dramatic findings stem from aggression within the Chester Zoo group and in spider monkeys from different zoological parks. Spider monkeys differ from most of their Old World counterparts because there is no evidence of clear-cut dominance relationships (Aureli & Schaffner, 2008), a hallmark of the social lives of many Old World primates (Kappeler & van Schaik, 2002). Results from the questionnaire found proportionally high incidences of male-male aggression which was surprising considering the reported strong bonds between males in the wild (Aureli & Schaffner, 2008). While total numbers of incidents of aggression were low compared to other primates, the incidences of severe aggression which resulted in injuries and even death were comparatively high. These more serious incidents were almost exclusively reported as being between males and of the six lethal incidents reported both actors and targets were male. Serious incidents of aggression are known to trigger a high stress response in other primate species (Eberhart, et al., 1983; Ostner, et al., 2008; Sapolsky, 1982; T. E. Smith & French, 1997b) and this was supported by my research which demonstrated that the highest levels of cortisol occurred in the aftermath of severe and lethal aggression. For severe aggression this led to short term high levels of urinary cortisol on the day following the conflict for the targets of aggression, whereas lethal aggression led to long-term elevations in cortisol for at least a week following the attacks. These levels of cortisol following aggression were four to seven times higher than the other stressful events I identified in my study.

That male-male aggression would be so prevalent and result in so many lethal events was not anticipated because the majority of the literature reports that male directed female aggression is the most frequent form of spider monkey aggression (Fedigan & Baxter, 1984; Slater, et al., 2009) and males have the strongest social bonds among the different age sex classes (Ahumada, 1992; Aureli & Schaffner, 2008). However, lethal male-male aggression has recently been reported in some of the long term field studies of spider monkeys (Campbell, 2006b; Valero, et al., 2006). This evidence seems to suggest a more complex relationship exists between the males and in particular the sub adult males who seem especially vulnerable to the lethal aspects of aggression. One interpretation of the male-male aggression observed in the wild is that it stems from competition for females and may be triggered by the operational sex-ratio in wild communities (Valero, et al., 2006).

Spider monkeys are unusual compared to most other monkey species in that the males remain in their natal group and are therefore likely to be closely related (Di Fiore & Campbell, 2007), while the females disperse. In the management of spider monkeys in zoological parks however it is common practice to move males between groups, despite the recognised importance of maintaining animals in zoological parks in natural social groupings and context (Carlstead, 1996; Robinson, 1998; WAZA, 2005). This practice may then exacerbate the natural tendency of males to target each other when reproductive competition is high. In fact, in the questionnaire study (Chapter 4), introducing males although infrequent was highlighted as being a significant catalyst for aggression and could be traced back to introducing unrelated males together in the same social group. This study therefore recommends that the transfer of young adult females should be considered instead of males as this replicates the natural dispersal patterns of spider monkeys in the wild. Identified problems with male-male aggression, even between related individuals, will however also need to be carefully monitored and managed.

Given the pattern of male-male aggression and the HPA response that spider monkeys experienced in the aftermath of severe and lethal aggression, I believed it was essential to capture the behavioural and physiological responses in the spider monkeys when Chester Zoo replaced their breeding male in 2008 (Chapter 6). Although the case study found a significant behavioural effect in the resident adult females, there was little evidence of an increase in urinary cortisol among the females. In addition, there were no instances of aggression between the adult male and juvenile male in the group. This could be because the new male on his arrival was not deemed a threat, and this was supported by the male showing no aggressive behaviour towards any of the resident animals throughout the study. There were also no other adult or sub adult males present during this introduction which otherwise may have been a source of tension. Unfortunately, there were insufficient samples taken from the new male to determine whether the introduction did cause a significant stress response in him, although there was some behavioural evidence.

The method of introduction may also have been significant in reducing the overall stress response. A gradual approach was adopted with the male being kept initially in a separated section of the indoor enclosure that allowed full visual and partial tactile contact with the rest of the group. This has been shown in other species to reduce the impact of an introduction and appeared to have also been successful in

reducing the overall stressful nature of the introduction (Alford, et al., 1995; Reinhardt, et al., 1995; Schaffner & Smith, 2005). This provides evidence of the successful introduction of a new male into a group and points to recommendations for managing captive spider monkey populations (see below 7.6).

Another unanticipated result was the change in cortisol levels observed in other adult females during the births. There seemed to be a definite pattern of an increase in the other females immediately following a birth. The reasons for this are unclear, but evidence from the field suggests new spider monkey mothers often spend much of their time alone (McFarland Symington, 1987), which may be an evolutionary trait to keep away from others who may see the infant as a future potential competitor for resources (Chapman, et al., 1989). The forced proximity with the restriction of area within a zoo environment reduces the opportunities for females to fission from the social group at this critical time and this may have been a potential stressor.

7.5 Assessing the welfare in zoo-housed spider monkeys

Three main strategies have been proposed for the assessment of welfare (see Chapter 1, section 1, 3). The assessment of welfare in zoo-housed spider monkeys in this study adopted measures of biological functioning (Barnett & Hemsworth, 1990; Duncan & Fraser, 1997). This was mainly by a longitudinal physiological approach with the measure of GCs recorded over a seven year period, although behavioural measures were key in the interpretation of the results. The use of physiological measures on their own can be difficult to interpret, but particularly over the long term can be an indicator as to how well an animal is coping within its environment (Barnett & Hemsworth, 1990; Lane, 2006). Such an approach has been recommended as a means of improving understanding of how to care for zoo animals, and in particular for identifying specific characteristics of a zoo environment that may have a significant impact on their lives (Carlstead & Shepherdson, 2000; Shepherdson, et al., 2004; Wielebnowski, 2003). Behavioural measures were also used to aid in the interpretation of the changes in cortisol and to identify particular behaviours or behaviour patterns that have been associated with a stress response. This study has contributed to the understanding of how well spider

monkeys adapt to a particular zoo environment and has highlighted potential factors which can result in a stress response, and therefore a potential negative impact on their welfare. It has also highlighted the usefulness of cortisol studies in their potential in contributing to the assessment of welfare of individual animals within a zoo environment.

Information on the natural behavioural repertoire of spider monkeys in the wild were also used as a means to identify their species-specific requirements and to help explain the unusual aggressive behaviour patterns recorded in zoological parks (Stolba & Wood-Gush, 1989; Veasey, et al., 1996b). An integrative approach using behaviours as well can help identify behaviour patterns that may be indicative of a stress response as was identified in the elevated scratching rates recorded in adult females during the introduction of the new male (Chapter 6).

Overall several stressors were identified and assessed using these procedures, and included zoo visitors, aggression, social separations, introductions and births. Although some of these events resulted in dramatic elevations in urinary cortisol in some individuals, they were not sustained. This suggests that although such events resulted in an acute stress response there was no clear evidence of chronic stress indicating that the spider monkeys at Chester zoo appeared to be able to adapt to their particular social and physical environmental. However there may still be a degree of individual variation which can be based on age, sex and experience. During the course of the study one individual Mil died from natural causes. However, she was a hand reared individual that appeared to be poorly integrated with other group members and for the year prior to her death had baseline cortisol levels three-fold higher than the baseline of other members. This sustained elevated level of cortisol may have placed her in a prepathelogical state (Moberg, 2000), which would have made her more susceptible to the viral infection that killed her.

7.6 Implications for other zoo studies

My findings add to the growing body of literature which indicates that urinary cortisol is an effective means of assessing the impact of environmental and social stressors within a zoological park setting (Carlstead & Shepherdson, 2000; Wielebnowski, 2003). Providing there is appropriate validation for each species

(Buchanan & Goldsmith, 2004), it can be used as a non invasive method to assess any potentially aversive impact on zoo animals. For example, to maintain genetic diversity the movement of animals across populations is essential, but it can be disruptive and cause social instability (Kleiman, 1980). This method can be used for monitoring the stress response to assess introductions and identify the least disruptive techniques. The activity of the HPA axis could also be used to assess visitor effect and other potential stressors within a zoo environment in a variety of taxa with important implications for the design of exhibits. An enclosure which allows the animals the opportunities to perform their natural behaviours will have positive consequences for their welfare, as well as education and conservation activities by zoological parks (Carlstead, 1996; WAZA, 2005). Finally such studies can add scientific evidence to husbandry requirements which are produced for many species of zoological park animals (Shepherdson, et al., 2004).

7.7 Further studies

Following the visitor effect on cortisol levels in the group of spider monkeys at Chester Zoo it would be important to also assess cortisol levels in other spider monkeys at other zoological parks, in particular in enclosures where there are not the opportunities to escape from visitors. A further study that validates more concretely a discrete behavioural measure of stress, such as scratching, would also be beneficial to allow for a more accessible and more cost effective method of assessing potential stressful events, such as introductions. More investigation is required into the effect of introductions of males into established groups and to assess various techniques of introduction. The assessment of female introductions would also be of interest to demonstrate whether introductions that reflect the natural dispersal pattern of spider monkeys are less stressful for the group and the individual. The measurement of GC levels in spider monkey groups in other zoological parks would provide additional evidence to assess whether enclosure characteristics may influence GC levels and help identify potential aversive factors which can then be investigated in order to improve their welfare. The measure of the GC response in other spider monkey relocations would also help identify whether there are any differences in age, sex or

specific techniques which may provide information on the suitability of individuals to introductions.

Longitudinal hormonal and behavioural monitoring, along with keeper and health records, can be also used to assess the effect of various environmental and social changes. Another possible area of further study would be to investigate how the zoo environment affects the welfare of other species of animals that also live in social systems that are normally characterised by high fission fusion dynamics such as chimpanzees (*Pan troglodytes*) (McFarland Symington, 1990), spotted hyena (*Crocuta crocuta*) (Holekamp, et al., 1997) and African elephants (*Loxodonta africana*) (Wittemyer, Douglas-Hamilton, & Getz, 2005).

7.8 Recommendations for management of zoo-housed spider monkeys

First, larger and more complex enclosures that allow opportunities for individuals to temporarily separate themselves from the larger social group, in order to simulate fission events in the wild, may reduce the frequency and severity of aggression and provide a means of retreating when zoo-visitor numbers are high (Caws, et al., 2008; Wehnelt, et al., 2006). This may be particularly important for spider monkeys as fissioning away from group members is an important strategy for coping with conflict in wild spider monkeys (Aureli and Schaffner, in prep; Rebecchini, Schaffner & Aureli, in prep).

The relocation of males rather than females should be adopted in order to follow the normal immigration patterns of wild spider monkeys in which females emigrate and males are philopatric (Di Fiore & Campbell, 2007; Fedigan & Baxter, 1984; Vick, 2008). The social and physical environments in which spider monkeys tend to be kept in zoological parks do not reflect that found in nature and may exacerbate the propensity for male-male aggression in the wild. Therefore, those managing zoo populations of spider monkeys should consider relocating females rather than males. Due to well known problems of introducing male chimpanzees into new groups the relocation of females is already a protocol followed with a different primate species that has a social organisation characterised by high fission-fusion dynamics (Carlson, 2006; Fulk, 2000).

Third, the technique of a gradual introduction although still eliciting an increase in GC levels for the resident females along with some associated behavioural changes seemed to follow previous studies in captive primates (Brent, et al., 1997; Reinhardt, et al., 1995) and reduced the overall impact of the introduction. By having the facility to separate individuals from a group, but to remain in full visual and tactile contact, helps facilitate what can be potentially stressful events such as introductions and should be recommended as a management technique for future introductions in spider monkey groups.

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APPENDICES

Appendix A – Template for cortisol assay

Cortisol #_____ Samples_____ Comments AB conc

Date: _____ Dilution_____ Incubation start time HRP conc

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	Bo	1000 pg	500	250	125	62.5	31.25	15.6	7.8	3.9	1.95	Bo
D												
E												
F	Bo	1.95	3.9	7.8	15.6	31.25	62.5	125	250	500	1000 pg	Bo
G												
H												

Appendix B – Template for creatinine assay

Creatinine #_____ Samples_____ Comments

Date: _____ Dilution_____

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	6 µg	3 µg	1.5 µg	0.75 µg	0.00	HQC	LQC				
B												
C												
D												
E												
F												
G												
H												

Appendix C - Questionnaire

March 6, 2002

To Whom It May Concern:

Enclosed you will find a survey that aims to investigate aggression in captive spider monkeys. Spider monkeys are somewhat understudied with respect to other species of monkeys, such as macaques. To better understand aggression in captive spider monkeys we are seeking assistance from other zoos and wildlife parks that house relatively large groups of spider monkeys (>3 individuals).

In the wild, spider monkeys form multi-male/multi-female societies in which the males form the core of the group and maturing females leave their natal group and immigrate to a new group. Within spider monkey society, which ranges from 18 to upwards of 70 individuals, the monkeys form subgroups, of approximately 3-5 individuals, and these sub-groupings are fluid as membership within the small groups is constantly changing (Fedigan & Baxter 1984; Chapman 1990; McFarland Symington 1990; Chapman et al. 1995).

In spite of this fission-fusion social organisation, which appears to reduce conflict among group members over limited resources, severe aggression by males toward females and juvenile males has been reported in wild populations. In order to better understand the relationship between social organization and aggression, as well as the implications for colony management and well-being of spider monkeys, we are seeking information on the patterning of aggression in captive spider monkeys (*Ateles* species) by asking keepers and other appropriate staff to fill out the attached survey.

Thank you for your time and effort involved in participating in my study. We will be disseminating a final report to all the institutions that participated. We would appreciate having the survey returned to Nick Davis at Chester Zoo by April 30, 2002. If you have any questions regarding the survey, feel free to contact Nick Davis at Chester Zoo (n.davis@chesterzoo.co.uk). The survey has been reviewed and endorsed by the studbook keeper for spider monkeys in Europe.

Sincerely,

Nick Davis, BSc, MSc
Primate Keeper
Chester Zoo
Upton, Chester
CH2 1LH

Colleen M. Schaffner, Ph.D.
Department of Psychology
Chester College
Chester
CH1 4BJ

Spider Monkey Survey

Our survey seeks information regarding specific incidents of aggression that resulted in physical injury to spider monkeys and the social organisation of the group at the time the aggression occurred. We appreciate the time commitment involved in filling out a survey of this scope and will provide all respondents with a summary of my findings. Any information that you can provide would be appreciated.

Name of your institution

_____

Contact person and number or

email_____

_____

Species of spider monkey: circle one

Ateles fusciceps *Ateles belzebuth* *Ateles geoffroyi* *Ateles paniscus*

1) Have you observed or recorded injurious aggression, aggression that required veterinary assistance (antibiotics, stitches, temporary isolation of individual), in your colony of spider monkeys during the past five years (if possible)?

Yes ☐ No ☐

If you have not witnessed or recorded injurious aggression in your spider monkey group, please return the survey indicating that your group is injurious aggression free. Please include any information about the social organisation as this information is also valuable.

2) In your experience, does any particular event or situation appear to lead to injurious aggression in your spider monkey group?

3) In your opinion does spider monkey aggression differ compared to other primate species you work with, and if so how does it differ? (for example, frequency, duration, intensity)

4) *For each instance of severe aggression in which animals required veterinary attention (e.g., received antibiotics or stitches) or other cases you feel would be of interest, please indicate the following points from a through g:

a) the date of observed aggression or injuries;
b) the composition of the group, indicating the sex and age of each member, their genetic relationship to one another (e.g. by producing a taxonomic report for the day of aggression);
c) whether there were any recent changes in group composition, if so what was the change;
d) which animals were injured (animal I.D. or indicate sex and age);
e) the injuries observed;
f) the aggressor (where known, animal I.D. or sex and age);
g) any additional information available regarding the incident.

*Please feel free to attach ARKS or MEDARKS database printouts in lieu of filling out this portion of the survey if this facilitates your ability to respond.

Dear

Following a questionnaire on aggression in spider monkeys which you completed back in 2002 I am after some further information which will help in the analysis of the results. It has been suggested that enclosure size may be an important factor in the rates of aggression in spider monkeys. I would be grateful if you could send me the approximate areas (m^2) of both the indoor and outdoor enclosures during the period 1997 to 2002.

Thank you again for your time and effort involved in participating in our study. We will be disseminating a final report to all the institutions that participated on completion. If you have any questions regarding the survey, feel free to contact myself at Chester Zoo (n.davis@chesterzoo.org). The survey has been reviewed and endorsed by the studbook keeper for spider monkeys in Europe

Regards

Nick Davis, BSc, MSc
Specialist keeper (projects)
Chester Zoo
Upton, Chester
CH2 1LH

Colleen M. Schaffner, Ph.D.
Department of Psychology
University of Chester
Chester
CH1 4BJ

Appendix D - Behaviour check sheet for introduction of new male

Date	Proximity	Proximity ID's	Focal Count	Focal ID	Focal Duration	Actor	Behaviour	Recipient	T Start	T Finish	Duration	Comments

Appendix E

Davis, N., Schaffner, C.M., & Smith, T.E. (2005). Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science* 90(2), 1331-141.

Appendix F

Davis, N., Schaffner, C.M., & Wehnelt, S. (2009). Patterns of injury in zoo-housed spider monkeys: A problem with males? *Applied Animal Behaviour Science* 116(2-4), 250-259.

Appendix G

LIST OF CONFERENCE PRESENTATIONS

Davis, N., Schaffner, C.M., & Smith, T.E. (2002). The impact of zoo visitors on hormonal indices of stress in spider monkeys (*Ateles geoffroyii fusciceps*). Paper presented at XIX International Primatological Society Congress, 5th – 9th August 2002, Beijing, China.

Davis, N., Schaffner, C.M., & Wehnelt, S. (2004). The context, direction and intensity of aggression in captive spider monkeys. Paper presented at XX International Primatological Society Congress, 23rd – 28th August 2004, Torino, Italy.

Davis, N., & Schaffner, C.M. (2005). Dynamics of aggression in zoo-housed spider monkeys. Paper presented at Spring Primate Society of Great Britain Meeting, 22nd – 23rd March 2005. University College Chester, UK.

Davis, N., Schaffner, C.M., & Smith, T.E. (2008). Evidence for aggressive conflicts leading to increased urinary cortisol in a zoo-housed group Of spider monkeys. Paper presented at XXII International Primatological Society Congress, 3rd – 8th August 2008, Edinburgh, UK.

Davis, N., Schaffner, C.M., & Smith, T.E. (2009). The impact of social events on urinary cortisol in zoo-housed spider monkeys. Paper presented at the Spring Primate Society of Great Britain Meeting, April 16th – 17th 2009, University of Bournemouth, UK.